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(54) Title: MICRODEVICE AND METHOD FOR DETECTING A CHARACTERISTIC OF A FLUID

(57) Abstract: A microdevice for supporting a flowing fluid is disclosed. In one embodiment, the microdevice includes a substrate and a pair of generally parallel, spaced wall members on the substrate. At least one of the wall members includes a pair of structures defining an opening.

MICRODEVICE AND METHOD FOR DETECTING A CHARACTERISTIC OF A FLUID

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application no. 09/353,554, filed July 14, 1999, which is a continuation-in-part of U.S. patent application no. 09/115,397, filed July 14, 1998. This application also claims the benefit of the filing date of U.S. provisional patent application no. 60/175,997, filed January 12, 2000. All of the above U.S. provisional and non-provisional applications are assigned to the same assignee and are all herein incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

Work is now underway to develop microfluidic devices for analyzing chemical or biological fluids. A "microfluidic" device typically includes fluid channels having microscale dimensions. For example, a fluid channel in a typical microfluidic device may have a width of less than about 1000 microns.

In a typical application for a microfluidic device, a fluid containing a chemical compound may flow towards a reaction site on the microfluidic device. At the reaction site, the fluid may contact another fluid containing a different substance. The characteristics of the resulting fluid passing downstream of the reaction site may be detected to determine if the chemical compound reacts with the substance. The characteristics of the fluid may correspond to, for example, the concentration of the chemical compound in the fluid stream. If the concentration of the chemical compound in the fluid passing downstream of the reaction site is lower than the concentration of the chemical compound upstream of the reaction site, then it is likely that the chemical compound reacts with the substance.

Microfluidic analytical systems have a number of advantages over other types of analytical systems. For example, microfluidic systems are particularly well suited for analyzing or reacting samples with low volumes. In a typical microfluidic system, samples on the order of nanoliters or even picoliters can be reacted or analyzed.

Because of the small volumes of fluids being handled, microfluidic analytical systems may be used to rapidly assay large numbers of samples. The assays can be performed to study the effect of numerous compounds in various biological processes. For example, test compounds that may block, reduce, or enhance the interactions between different biological molecules, such as a receptor molecule and a corresponding ligand, may be identified as potential candidate drugs.

In recent years, the number of test compounds produced by modern combinatorial chemistry techniques has dramatically increased. While conventional microfluidic systems can be used to test the increasing number of compounds, the throughput of such systems could be improved. There is a continuing need to screen large numbers of samples quickly and accurately.

Embodiments of the invention address this and other problems.

SUMMARY OF THE INVENTION

Embodiments of the invention can be used to quickly detect the characteristics of fluids in a microdevice. Embodiments of the invention can be used for, for example, high-throughput drug candidate screening and medical diagnostics.

One embodiment of the invention is directed to a microdevice for supporting a flowing fluid. The microdevice comprises: a substrate; and a pair of generally parallel, spaced wall members on the substrate, wherein at least one of the wall members includes a pair of structures defining an opening.

Another embodiment of the invention may be directed to a microdevice comprising: a substrate; a plurality of wall members; and a plurality of fluid channels, wherein each of the fluid channels is defined by adjacent wall members in the plurality of wall members, wherein each wall member comprises an opening that is formed by opposed beveled structures of the wall member and that communicates the adjacent fluid channels.

Another embodiment of the invention is directed to a method for detecting a characteristic of a fluid, the method comprising: (a) inserting a probe into a fluid channel in a microdevice; (b) detecting a characteristic of a first fluid flowing in the first fluid channel; (c) moving the probe from the first fluid channel through an opening in one of the walls defining the first fluid channel and to a second fluid channel adjacent to the

first fluid channel; and (d) detecting a characteristic of a second fluid flowing through the second fluid channel.

Another embodiment of the invention is directed to an analytical assembly comprising: a detection assembly comprising a plurality of detection devices; and a microdevice comprising a plurality of wall members and a plurality of fluid channels, wherein each of the fluid channels is defined by adjacent wall members in the plurality of wall members.

Another embodiment of the invention is directed to a method for detecting a characteristic of a fluid, the method comprising: flowing a plurality of different fluids through respective fluid channels in a microdevice, each of the fluid channels in the microdevice being formed by adjacent pairs of wall members; and detecting characteristics of the plurality of different fluids substantially simultaneously using a plurality of detection devices as the different fluids flow through their respective fluid channels.

These and other embodiments of the invention are described in further detail with reference to the Figures and the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a top view of a microdevice according to an embodiment of the invention.

FIG. 2 shows a side view of the microdevice shown in FIG. 1 along the line 2-2.

FIGS. 3(a)-3(c) show partial top views of portions of wall members with beveled ends.

FIG. 4 shows an end cross-sectional view of the microdevice shown in FIG. 1 along the line 4-4.

FIG. 5 is a cross-sectional view of the microdevice shown in FIG. 1 along the line 5-5.

FIG. 6 is a side cross-sectional view of an analytical system shown in FIG. 7 along the line 6-6.

FIG. 7 is a top cross-sectional view of some components of an analytical assembly according to an embodiment of the invention. Boundaries forming a slot in a cover are shown by dotted lines.

FIG. 8 is a side cross-sectional view of an analytical assembly according to an embodiment of the invention.

FIG. 9 is a side cross-sectional view of an analytical assembly according to an embodiment of the invention.

FIG. 10 is a top cross-sectional view showing some components of an analytical assembly according to an embodiment of the invention.

FIG. 11 is an end cross-sectional view of an analytical assembly shown in FIG. 10 along the lines 11-11. Invisible lines show boundaries of a slot in a cover.

FIG. 12 is a schematic diagram of an analytical assembly embodiment.

FIG. 13 is a top view of an analytical assembly according to an embodiment of the invention.

FIG. 14 is a graph of surface potential vs. time as a probe scans fluids flowing in fluid channels in a microdevice according to an embodiment of an invention.

DETAILED DESCRIPTION

Embodiments of the invention can be used to rapidly detect characteristics of a plurality of different fluids. The fluids may be gases or liquids. Exemplary liquids include biological fluids such as blood or urine, cell extracts, organic fluids, solvents, aqueous solutions, and the like. Exemplary gases include air samples, hydrocarbon gases, etc. Regardless of the form of the fluids, the fluids may comprise atoms, organic or inorganic molecules such as proteins, organelles such as cells, and the like.

The different fluids flow through a plurality of different fluid channels at a detection region of a microdevice. The different fluids may have distinct characteristics and may be the products of events that occur before the different fluids flow through the detection region of the microdevice. For example, the different fluids may be downstream products of upstream events such as potential or actual interactions between substances. Events may include chemical or biological reactions between two substances and binding events between two substances.

Downstream of the events, characteristics of the fluids can be detected at the detection region of the microdevice. The characteristics of the fluids that are detectable may be either quantitative or qualitative in nature. In some embodiments, characteristics of the fluids such as emitted radiation (e.g., light), conductivity, the pH and the like of the different fluids flowing in the different fluid channels can be detected to

analyze the different fluids. Such characteristics may correspond to the types and/or amount of substances in the fluids. In some embodiments, the detected characteristics may serve as a direct or an indirect indicator of the concentration or amount of a particular substance in the fluid. For example, solutions containing protons are conductive. The conductivity or resistance of a fluid may be an indirect indicator of the concentration of protons in the fluid.

Interactions that can be assayed according to embodiments of the invention may be any type of interaction normally observed for biological moieties including, for example, a catalytic reaction of an enzyme, a binding event, or a translocation by a membrane protein through a lipid bilayer. In embodiments of the invention, separate fluid samples can be screened for their ability to interact with a biological moiety. For example, different fluid samples containing respectively different substances can flow through separate fluid channels in a microdevice and can be delivered to separate reaction sites on the microdevice. Each of the reaction sites may comprise an immobilized biological moiety, and the immobilized moieties may be bound to respective surfaces of different fluid channels. At the reaction sites, the biological moieties may or may not interact with the different fluid samples. Downstream of the reaction sites, the characteristics of the different fluids may be detected, either directly or indirectly to determine if any of the fluids of the substances in the different fluids have interacted (e.g., by binding together) with the immobilized biological moiety at each reactive site. For example, one or more detection devices downstream of the reactive sites may measure the concentration of the different substances in the fluids passing downstream of the reaction sites by detecting characteristics of the fluids. If the concentration of a substance in a fluid passing downstream of a reaction site is less than the concentration of the substance in a fluid upstream of the reaction site, then it is likely that the substance in the fluid is interacting (e.g., binding or reacting) with the immobilized biological moiety. On the other hand, if the concentration of a substance in a fluid downstream of the reaction site is substantially equal to the concentration of the substance upstream of the reaction site, then it is likely that little or no interaction is occurring between the substance in the fluid and the immobilized biological moiety.

In another example, upstream events may be specific conditions that are applied to different fluids in the different fluid channels to see if the fluids or substances in the fluids change as a result of the conditions. For instance, a plurality of different fluids may be subjected to different heating, cooling, and irradiation (e.g., with light)

conditions. Characteristics in the fluids passing downstream of these events may be detected to determine if the conditions affect the fluids.

In some embodiments of the invention, characteristics of the different fluids in the fluid channels may be detected by using a probe. The probe may pass through a plurality of different fluids in respective fluid channels by passing through openings in wall members that define the fluid channels. The characteristics of the fluids in these fluid channels can be quickly detected without exposing the end of the probe to an environment outside of the flowing fluid.

In other embodiments of the invention, a plurality of detectors may detect characteristics of a plurality of fluids flowing through a plurality of fluid channels in a microdevice substantially simultaneously. A detection assembly comprising multiple detectors may be used to detect the characteristics of the fluids flowing in the fluid channels substantially simultaneously. In these embodiments, the wall members defining the plurality of fluid channels may or may not have openings.

These and other embodiments are described in further detail below.

I. Embodiments using microdevices

One embodiment of the invention is directed to a microdevice. The microdevice may include a plurality of fluid channels defined by a plurality of wall members. The plurality of wall members may include at least one wall member having at least one opening that communicates two adjacent fluid channels. An opening in the wall member may be formed by opposing beveled structures at the internal ends of portions of the wall member. In embodiments of the invention, different fluids flowing in the adjacent fluid channels may have a laminar profile and do not mix in an appreciable manner as they flow past the opening and contact each other at the opening. Intermixing between the contacting fluids is minimal, even though there is no physical barrier in the wall member at the opening.

When openings in the respective wall members in the microdevice are aligned, a slot may be formed by the aligned openings. A probe disposed in a fluid in a fluid channel can move laterally through the slot and from fluid channel to fluid channel. For example, the probe can contact a fluid in a fluid channel and can detect a characteristic of that fluid. The probe can then pass through an opening in a wall member defining the fluid channel to an adjacent fluid channel where a characteristic in the

adjacent fluid channel may be detected. By analyzing different fluids in this manner, characteristics of the different fluids in the fluid channels can be quickly and accurately detected by the probe and subsequently analyzed. For example, in some embodiments, the characteristics of ten different fluids flowing in the different fluid channels may be accurately detected in less than one minute.

Illustratively, a probe for a pH sensor may be placed in a fluid channel to detect the pH of the fluid in that channel. Then, the probe can move laterally from one fluid channel to another adjacent fluid channel through the opening in a wall member disposed between these two fluid channels. The lateral movement of the probe can take place without withdrawing the probe from the fluids. Once the probe is in contact with the fluid in the adjacent channel, the pH of the fluid in the adjacent channel can be detected. This process can be repeated as the probe moves through the slot formed by the aligned openings in the wall members.

Embodiments of the invention provide a number of advantages. For example, in embodiments of the invention, a probe can pass through a number of fluid channels and can detect characteristics of the fluids in the fluid channels quickly and accurately. The probe need not be withdrawn from the fluid flowing in a channel and then inserted into an adjacent fluid channel. The distance that the probe travels between adjacent fluid channels is minimized thus reducing the time needed to analyze the fluids flowing in the microdevice. Moreover, since a probe need not be withdrawn from a fluid, the probe need not be aligned in a z-direction (i.e., relative to a x-y plane formed by the orientation of the microdevice) as it moves from fluid channel to fluid channel. The z-direction alignment step takes time and increases the chance of damaging the probe. For example, if a probe is inserted too far into a fluid channel, the probe may contact the fluid channel bottom surface potentially damaging the probe. In embodiments of the invention, the probe can be aligned in the z-direction once. To detect the characteristics of other fluid streams, the probe may move in an x- or y- direction while remaining a predetermined distance above the fluid channel bottoms. Also, by keeping the probe at a substantially constant z position, the reliability of measurements conducted by the probe can be improved in some instances. For example, the characteristics of a fluid flowing in a fluid channel may be a function of insertion depth in a fluid. Keeping a probe at a substantially constant z position when detecting characteristics of multiple fluids can eliminate any potential variation in any detected characteristics that may be due to different probe insertion depths. Furthermore, in embodiments of the invention, purging

is not required betw en two successive detections (e.g., two successive measurements). In some conventional microfluidic devices, different fluids to be analyzed pass through a single fluid channel. Purging fluids are needed to separate the different fluids as they flow in series through the fluid channel. However, in embodiments of the invention, different fluids may flow in different, parallel fluid channels at a detection region in the microdevice. The fluids in the different fluid channels may be detected in series or in parallel without using purging fluids. Furthermore, the microdevice embodiments of the invention are especially suitable for use with biosensors. Typical biosensors may contain biological molecules such as lipids, enzymes, or receptors. If biological molecules such as these are exposed to air, they may become inactive. Moreover, a typical biosensor may have a variable "wetting" period after a sample fluid is applied to the biosensor. In embodiments of the invention, a probe can pass between different fluid streams without exposing the probe to an external environment such as air. Accordingly, the microdevice embodiments of the invention are especially useful for containing fluids that are to be analyzed using a biosensor. In addition, since fluid streams can contact each other yet not mix in an appreciable manner in embodiments of the invention, reactions at the interface of two flowing fluids may be analyzed. One or more probes may detect the characteristics of a fluid passing downstream of the interface of the two flowing fluids to study the interaction between the two fluids.

A microdevice embodiment is shown in FIG. 1. FIG. 1 shows a microdevice **10** comprising a substrate **12**, a plurality of inner wall members **14a-14e**, and a plurality of outer wall members **14o, 14p**. The plurality of inner wall members **14a-14e** is disposed between the outer wall members **14o, 14p**. Both the inner wall members **14a-14e** and the outer wall members **14o, 14p** are disposed on the substrate **12**. In this example, the inner wall members **14a-14e** and the outer wall members **14o, 14p** are substantially parallel to each other.

The wall members **14a-14e, 14o, 14p** are spaced so that each pair of adjacent wall members **14a-14e, 14o, 14p** produces a fluid channel **16a-16d**. For example, adjacent inner wall members **14a, 14b** produce an inner fluid channel **16a**. The inner wall members **14a, 14e** and outer wall members **14o, 14p** form outer fluid channels **16o, 16p**. For example, inner wall member **14a** and outer wall member **14p** form a fluid channel **16p**. FIG. 2 shows a side view of the outer wall member **14o** and the substrate **12** of the microdevice **10**. In this example, the outer wall member **14o** is solid along its length and does not have an opening like the inner wall members **14a-14e**.

The fluid channels **16a-d, 16o, 16p** in the microdevice **10** shown in FIG. 1 are substantially parallel to each other. However, in other embodiments of the invention, the fluid channels and the wall members forming those fluid channels may have any suitable configuration. For example, the fluid channels in the microdevice may be fabricated so that they are perpendicular or non-linear. Moreover, while the microdevice **10** shown in FIG. 1 has six fluid channels, it is understood that in embodiments of the invention, the microdevice **10** may have any suitable number of fluid channels. For example, in some embodiments, the microdevice **10** may have more than 10, 20 or 50 fluid channels.

Each inner wall member **14a-14e** can structurally discontinue at an intermediate region to form an opening **20a-20e**. Although the embodiment shown in FIG. 1 has one opening **20a-20e** per wall member **14a-14e**, it is understood that embodiments of the invention are not limited to microdevices with one opening per wall member. For example, each wall member may have 2, 3, 4, or any suitable number of openings. Moreover, as will be explained in further detail below, in some embodiments, the wall members need not have any openings in them.

In some embodiments, the openings **20a-20e** in the members **14a-14e** may be aligned to form a slot **140**. The slot **140** formed by the aligned openings **20a-20e** can, for example, permit a probe (not shown) to pass from one fluid channel to another fluid channel without being removed from the microdevice **10**. Illustratively, a probe (not shown) can detect a characteristic of a first fluid flowing in a first fluid channel **16a**. After detecting the characteristic, the probe may move through the opening **20b** and into a second fluid channel **16b**. The probe may then detect a characteristic (e.g., pH, conductivity, fluorescence, and/or temperature) in a second fluid flowing in the second fluid channel **16b** without removing the probe from the microdevice **10**. Fluids in the other fluid channels **16c, 16d, 16o** may be detected in a similar manner. The probe need not be withdrawn from the fluids flowing in the fluid channels **16a-16d, 16o, 16p** and need not be exposed to the outside environment. By detecting the characteristics of fluids in this manner, detection occurs quickly and accurately.

Each inner wall member **14a-14e** can include one or more pairs of opposing beveled structures **24a-24e** that form openings **20a-20e** in the respective wall members **14a-14e**. By using beveled structures in a wall member, a fluid having a laminar profile flowing in a fluid channel formed by the wall member is more likely to retain its laminar profile at the opening formed by the beveled structures. The beveled

structures **24a-24e** may have any suitable geometry. For example, two examples of beveled structures **24a** are shown in FIGS. 3(a), 3(b).

In FIG. 3(a), a wall member **14a** includes a beveled structure **24a**. The beveled structure **24a** includes a pair of tapering walls **28a**. In this example, the tapering walls **28a** are substantially straight. Also, the tapering walls **28a** converge in an inward direction to an apex **30** and may form an angle with respect to substantially parallel side surfaces **114a** of the wall member **14a**. The angle may be, for example, from about 1 degree to about 89 degrees. In other embodiments, the angle may be, for example, about 2 to about 20 degrees.

FIG. 3(b) shows another example of a beveled structure **24a** of a wall member **14a**. The beveled structure **24a** also has a pair of tapering walls **28a** that converge to an apex **30**. However, unlike the embodiment shown in FIG. 3(a), the beveled structure shown in FIG. 3(b) has curved tapering walls **28a**. In this example, the tapering walls **28(a)** curve inwards towards the apex **30**. The beveled structure **24a** shown in FIG. 3(b) has a generally funnel-shaped appearance when viewed from the top. The beveled structure **24a** shown in FIG. 3(c) is similar to the previously shown beveled structures, but includes a smooth transition between the side surfaces **114(a)** and the tapering walls **28(a)**. As shown, side surfaces **114(a)** may be substantially parallel to each other and may then gradually curve inwardly in the region of the tapering walls **28(a)**.

The particular geometries of the features of the microdevice **10** may vary. Examples of features include wall member thicknesses, fluid channel heights, and fluid channel widths. Typically, the features of the microdevice **10** have at least one dimension that is less than about 1000 microns. For example, in some embodiments, the width and depth of each fluid channel may be between about 10 microns and about 500 microns. In other embodiments, the width or depth of each fluid channel may be between about 50 microns and about 200 microns. In some embodiments, the fluid channels may sometimes be referred to as "microchannels".

Referring to FIG. 4, each wall member **14a-14e, 14o, 14p** may have a width "W" of less than about 1 mm (e.g., about 20 microns to about 100 microns) and a height "D" of less than about 1 mm. In some embodiments, D may be from about 50 microns to about 500 microns (e.g., about 200 microns). Each fluid channel **16a-16d, 16o, 16p** may have a width "w" of less than about 1 mm (e.g., about 50, 100, 150, or 200 microns).

Referring to FIG. 5, the distance "G "of each opening **20a** formed in a wall member **14a** may be about 1 mm or less. For example, in some embodiments, G may be from about 50 microns to about 400 microns (e.g., about 200 microns). As shown in FIG. 5, the wall member **14a** structurally discontinues to form an opening **20a** so that the wall member **14a** has two distinct, separated portions. Each portion of the wall member **14a** may have two parts. One part may have substantially parallel sidewalls and may have a length "L1" or "L2". The other part may be a beveled structure that extends along the length of the wall member **14a** a distance "S". Typically, the distance L1 or L2 is much greater than the length S. For example, the distance L1 or L2 may be about 1 cm or more (e.g., about 1 cm to about 5 cm). The length S may be about 50 microns to about 750 microns. Of course, the dimensions of the elements of the microdevice **10** may have values that are more or less than the specifically mentioned values.

Again referring to FIG. 1, the fluid channels **16a-16d, 16o, 16p** may have any suitable length or configuration. The length of each fluid channel **16a-16d, 16o, 16p** may be from about 1 to about 20 mm in length, or more. For example, the length of each fluid channel **16a-16d, 16o, 16p** can be from about 2 to about 8 mm. The distance between the corresponding points (e.g., opposing apexes) of opposed beveled structures in a wall member may be between about 50 and about 500 microns in some embodiments. Any channel cross-section geometry (trapezoidal, rectangular, v-shaped, semicircular, etc.) can be employed in the microdevice **10**. Trapezoidal or rectangular cross-section geometries may be used in the fluid channels **16a-16d, 16o, 16p**. Such geometries may be used with standard fluorescent detection methods.

Fluids such as liquids or gases may be supplied to the microdevice **10** in any suitable manner. For example, bulk-loading dispensing devices can be used to load all fluid channels **16a-16d, 16o, 16p** of the microdevice **10** at once with the same or different fluids. Alternatively, integrated or non-integrated microcapillary dispensing devices may be used to load fluids separately into each fluid channel **16a-16d, 16o, 16p** of the microdevice **10**.

The flow of the fluids within the fluid channels **16a-16d, 16o, 16p** can be controlled by the selective application of voltage, current, or electrical power to the substrate to induce and/or control the electrokinetic flow of the fluids. Alternatively or additionally, fluid flow may be induced mechanically through the application of, for example, differential pressure or acoustic energy to a fluid. Such fluid flow control mechanisms are used in microfluidic devices and are known in the art.

As noted, each of the fluids flowing in the fluid channels **16a-16d, 16o, 16p** may have a laminar profile. In this regard, the Reynolds number, Re, for the fluid streams in the fluid channels **16a-16d, 16o, 16p** may be greater than 0 to less than or equal to about 2300. Preferably, Re is from about 100 to about 2000. Re may be defined as follows:

$$Re = \frac{pV_{ave}D_h}{\mu}$$

p is the density in gm/cm^3, μ is viscosity in gm/cm·sec, V_{ave} is the average velocity of the fluid, and D_h is the hydraulic diameter. The hydraulic diameter, D_h, may be defined as follows:

$$D_h(cm) = \frac{4xCross - SectionArea\left(cm^2\right)}{Wetted\ Perimeter\ (cm)}$$

Although the fluids in the channels preferably have a laminar profile, adjacent fluids flowing in adjacent fluid channels may slightly intermingle (e.g., by diffusion) via the opening that communicates the adjacent fluid channels. However, the degree of intermingling between fluids in adjacent fluid channels does not typically interfere with any measurements or detections made by a probe.

Although many of the previously described examples have different sample fluids flowing through the fluid channels **16a-16d, 16o, 16p** in the microdevice **10**, in other embodiments of the invention, non-sample fluids such as wash fluids may be included in one or more of the fluid channels **16a-16d, 16o, 16p**. For example, a wash fluid that can be used to wash a probe may flow through one or more fluid channels **16a-16d, 16o, 16p**. For example, a fluid channel **16c** containing a wash solution is disposed between two fluid channels **16b, 16d** containing sample fluids. A probe (not shown) may be inserted into the fluid channel **16b** to detect a characteristic of a sample fluid flowing in the fluid channel **16b**. To detect a characteristic, the probe may be, for example, positioned in fluid channel **16b** between the openings **20b, 20c** or may be upstream or downstream of the point between the openings **20b, 20c**. After detecting the characteristic, the probe may pass through the opening **20c** in the wall member **14c** to the fluid channel **16c** containing a wash fluid. In the fluid channel **16c**, the wash fluid removes any materials that may be disposed on the probe and that may impede the probe's ability to detect a characteristic in a different fluid. After the probe is washed, the

washed probe may pass through the opening **20d** in the wall member **14d** to the other fluid channel **16d** containing the other sample fluid. The washed probe can then detect a characteristic of the sample fluid in the fluid channel **16d**. Alternatively or additionally, one or more of the fluid channels **16a-16d, 16o, 16p** may contain a calibration fluid that can be used to calibrate, for example, a probe. The probe can be calibrated while being disposed in a calibrating fluid and may move to a fluid channel with a sample fluid after the probe is calibrated.

FIG. 6 shows an analytical assembly comprising a probe assembly **46** and a microdevice **10**. The microdevice **10** in FIG. 6 is similar to the previously described microdevice **10** shown in FIG. 1, except that the microdevice **10** shown in FIG. 6 includes a cover **36**. The cover **36** may also comprise a plurality of fluid inlets (not shown) and a plurality of fluid outlets (not shown) that provide fluids to and remove fluids from the fluid channels **16a-16d, 16o, 16p** in the microdevice **10**.

The cover **36** is supported by the pair of outer wall members **14o, 14p** and may include a slot **40**. A pair of opposed, generally parallel, boundaries may define the slot **40** in the cover **36**. When the cover **36** is disposed on the wall members, the slot **40** in the cover **36** is aligned with and disposed over the slot **140** formed by the holes **20a-20e** in the inner wall members **14a-14e** (see FIG. 1). The boundaries defining the slot **40** in the cover **36** may or may not be generally aligned with apexes of the beveled structures in the wall members **14a-14e**. A probe **44** of a probe assembly **46** is inserted through the slot **40** in the cover **36** so that an end portion **47** of the probe **44** is disposed in a fluid channel **16a** and in the slot **140** in microdevice **10**.

In the analytical assembly shown in FIG. 6, the probe **44** may include an intermediate portion **45** that is upright and an end portion **47** that is skewed with respect to the intermediate portion **45**. The end portion **47** of the probe **40** may be substantially perpendicular to the intermediate portion **45**. In other embodiments, the end portion of the probe need not be perpendicular to an intermediate portion of the probe. For example, in some embodiments, the end portion of a probe may be co-linear with an intermediate portion of the probe.

In this example, the end portion **47** of the probe **44**, is directed towards the upstream direction of the fluid flowing (which flows in direction A) through the fluid channel **16a**. As the fluid flows through the fluid channel **16a**, the end portion **47** of the probe **44** may receive some of the fluid flowing in the fluid channel **16a**. Once the fluid is received, the end portion **47** may remove a portion of the fluid for sampling. For

example, the probe 44 associated with the probe assembly 46 may include a micro-pipe that collects some of the fluid flowing through the fluid channel 16a. Once collected, the sample may then be transferred to a mass spectrometer, HPLC (high pressure liquid chromatography) apparatus, or a gas chromatography apparatus. In some embodiments, the micro-pipe could also be used to introduce a fluid into a fluid channel. The introduced fluid can be added to a fluid channel without disturbing the laminar flow profile in the flowing fluid. Other suitable detection assemblies, detection devices, and analytical systems according to embodiments of the invention are described in further detail below.

Referring to FIGS. 6 and 7, to move the probe 44 from fluid channel to fluid channel, the probe 44 may move in the desired direction in the slot 40, such as in direction of arrow B (see FIG. 7). Because the end portion 47 in this example protrudes from the upright portion 45 of the probe 44, in order to pass the end portion 47 through the slot 40, the end portion 47 may be initially aligned with the slot 40 and may then be inserted through the slot 40 in the cover (not shown in FIG. 7). Once the end portion 47 is in the slot 140 formed by the openings 20a-20e in the wall members 14a-14e, it is rotated about 90° in direction of the arrow C shown in FIG. 7 so that the end portion 47 is directed toward the flowing fluid in the fluid channel in which it is disposed. The boundary 40a at slot 40 may be aligned with the apexes 30a-30e of the wall members 14a-14e so that the end portion 47 of the probe 40 does not contact the apexes 30a-30e as the probe 44 is inserted into the slot 40.

FIG. 8 shows another analytical assembly embodiment of the invention. In this embodiment, the microdevice 10 includes a cover 36 having slot 40. A lid 50 is on the cover 36 and is spaced from the cover 36 by supports (not shown). The slot 40 in the cover 36 is defined by downwardly sloping planar surfaces from boundaries 40a and 40b that terminate in edges 58a and 58b, respectively. The lid 50 also has a slot 60 that is generally aligned with the slot 40 in the cover 36. The probe 44 may pass through both the slot 60 in the lid 50 and the slot 40 in the cover 36.

The embodiment shown in FIG. 8 can be used when the fluids flowing through the fluid channels are gases. As gases flow through the fluid channels defined by the wall members and the substrate, another gas such as an inert gas (e.g., a noble gas, nitrogen, etc.) flows between the lid 50 and cover 36. The inert gas may flow in a direction of the arrow D and may have a higher pressure than the gases flowing through the fluid channels formed by the wall members on the substrate 12. The higher pressure

gas flowing between the lid **50** and the cover **36** confines gases flowing in the fluid channels between the cover **36** and the substrate **12** and prevents diffusion of the same out of the fluid channels and into the zone between the lid **50** and the cover **36**. In the embodiment shown in FIG. 8, the probe assembly **46** has a probe **44** with a beveled end and not a protruding end portion as in the previous examples. The probe assembly in the embodiment shown in FIG. 8 could also have a protruding end portion if desired.

FIG. 9 shows another analytical assembly embodiment of the invention. The microdevice **10** in this embodiment has a substrate **12**, a bottom member **80**, a slide member **90**, a cover **36**, and a probe assembly **46**, and a probe **44**. The bottom member **80** has a passage **82** where the slide member **90** is disposed. The slide member **90** may slide in a direction transverse to the orientation of the fluid channels **16a-16e, 16o, 16p** (i.e., in direction of the arrow **E** in FIGS. 10 and 11). As shown in FIGS. 10 and 11, the substances **94** disposed on the slide member **90** may be aligned with the fluid channels **16a-16e, 16o, 16p** so that the fluids flowing within the fluid channels **16a-16e, 16o, 16p** come in contact with the substances **94**.

Illustratively, with reference to FIG. 9, the slide member **90** may support substances **94** that can contact a fluid flowing through the fluid channel **16a** prior to reaching the probe **44** of the probe assembly **46**. The characteristic of the fluid in the fluid channel **16a** can be detected after the fluid has contacted the substances **94** on the slide member **90**. For example, the substances **94** may comprise antibodies for capturing molecules contained in the fluid flowing in the fluid channel **16a**. The probe **44** may then contact the downstream fluid and the probe **44** can detect a characteristic of the downstream fluid. The concentration of the molecules in the fluid can then be determined. If the concentration of the molecules upstream of the slide member **90** is greater than the concentration of the molecules downstream of the slide member **90**, then it can be concluded that the substances **94** on the slide member **90** interact with the molecules in the fluid.

In some embodiments, the microdevice **10** can be used to deposit successive layers of material on a slide member **90**. This may be done by pulling the slide member **90** through the passage **82** in the microdevice **10**. The slide member **90** may be exposed to a succession of many different fluids that may deposit different materials on the slide member **90**.

II. Detection assemblies and analytical systems

The detection methods, detection assemblies, and analytical systems used in embodiments of the invention are not limited to those described above, and may employ any suitable optical, electrical, physical, and/or chemical detection techniques. Radiation such as visible, infrared, or ultraviolet radiation from the fluids may be detected by a detection assembly being an optical detection assembly.

In many of the embodiments described above, detection assemblies and analytical systems using probes that comprise micropipes are described in detail. However, embodiments of the invention are not limited to the use of such micropipes. For example, the end portion of a probe may contact the fluid flowing in a fluid channel to detect a particular characteristic of the fluid, without collecting a sample of the fluid. The probe may be coupled to signal analyzer (such as that sold by Hewlett-Packard, for example), an oscilloscope (such as that sold by Tektronix or Hewlett-Packard), or a lock-in amplifier (such as that commercially employed by Stanford Research System or EG&G).

The probe may comprise a physical sensor, a biological sensor, a chemical sensor, or an electrical sensor. Examples of physical sensors include thermocouples, pressure sensors, flow sensors, optical fibers, etc. Examples of biological sensors include sensors with immobilized enzymes or immunoassays. Examples of electrical or chemical sensors include sensors with interdigitated electrodes having optional polymer coatings, atomic force microscopes (AFMs), Ion Sensitive Field Effect Transistors (ISFETs), light addressable potentiometric sensors (LAPSs), pH meters, and scanning probe potentiometers (SPPs). These and other types of sensors are described in Manalis et al, *Applied Physics Letters*, Volume 76, No. 8, February 21, 2000, and other references. In comparison to optical detection devices, chemical sensors and electrical sensors are desirable as they do not need to use more expensive and inconvenient fluorescent or radiochemical tagging processes.

An atomic force microscope allows high force sensitivity mapping of biological cells and molecules such as DNA and proteins. The AFM can obtain stable images of individual biomolecules while operating in physiological environments. In an AFM, unlike optical detection devices, molecules can be imaged directly, and the dimensions of the probe can determine the spatial resolution.

Field effect devices such as the ISFET and the LAPS can directly detect molecular and ionic charge. For example, the LAPS device has been used in a microphysiometer to monitor the response of cells to chemical substances by measuring the rate of change of the pH as protons are excreted from cells during metabolism. LAPS devices may be commercially obtained from Molecular Devices of Sunnyvale, CA.

Preferably, the active areas of electrical detection devices such as AFMs, ISFETs, and SPPs are small. In some embodiments, the active area in such detection devices is less than about a square millimeter, or less than 100 square microns. When the active area is small, the detection sensitivity and resolution is improved in comparison to detection devices with larger active areas.

Other detection devices may be used instead of or in addition to one or more probes. In some embodiments, detection devices such as one or more optical detection devices may be used to detect the characteristics of fluids flowing in the fluid channels in a microdevice. For example, FIG. 12 shows a schematic diagram of an analytical assembly comprising a detection assembly that detects fluorescent light coming from the fluids on a microdevice. In the illustrated detection assembly, the microdevice **121** is positioned on a base plate **120**. Light from a 100W mercury arc lamp **125** is directed though an excitation filter **124** and onto a beam splitter **123**. The light is then directed through a lens **122**, such as a Micro Nikkor 55 mm 1:2:8 lens and onto the fluids flowing in the fluid channels of the microdevice **110**. Fluorescence emission from the device returns through the lens **122** and the beam splitter **123**. After also passing though an emission filter **126**, the emission is received by a cooled CCD camera **127**, such as the Slowscan TE/CCD-10245F&SB (Princeton Instruments). The camera **122** is operably connected to a CPU **128**, which is, in turn, operably connected to a VCR **129** and monitor **130**.

In some embodiments of the invention, the analytical assembly may comprise a detection assembly comprising a plurality of detection devices and a microdevice. The microdevice may comprise a plurality of wall members and a plurality of fluid channels, wherein each of the fluid channels is defined by adjacent wall members in the plurality of wall members. The analytical assembly may be used to detect characteristics of different fluids flowing in different fluid channels substantially simultaneously. In these embodiments, the wall members of the microdevice may or may not have openings that allow adjacent fluid channels to communicate with each other. By using multiple detection devices, the characteristics of fluid flowing in the fluid channels

of a microdevice may be detected in parallel, thus increasing the speed of detection and analysis.

In one example, a plurality of different biological moieties can be screened in parallel for their ability to interact with a component of a fluid sample. A fluid sample can be delivered to the reactive sites in fluid channels in a microdevice where each of the different biological moieties is immobilized on a different site of the microdevice. Then, characteristics of the fluids passing downstream of the reactive sites may be detected substantially in parallel with a plurality of detection devices to study the interaction of the component with the immobilized biological moieties at each reactive site.

Illustratively, referring to FIG. 13, a slide member **90** may comprise a number of detection devices such as sensors **194** and may form a detection assembly. The sensors **194** on the slide member **90** may contact the fluids flowing in the fluid channels **16a-16e, 16o, 16p** and may subsequently detect characteristics of the fluids. The sensors **194** may be, for example, conductivity sensors, biosensors, temperature sensors, etc. In these embodiments, a probe assembly with an elongated probe, and a cover with a slot for the elongated probe are not needed.

Other detection assemblies with multiple detection devices may be used in embodiments of the invention. For example, probe assemblies like the probe assembly **46** shown in FIG. 6 can be used. The probe assembly, however, may comprise two or more elongated probes **44**. These probes may be spaced so that they can be inserted into plural fluid channels simultaneously to detect characteristics of the fluids flowing in these fluid channels substantially simultaneously. In some embodiments, the number of probes in the detection assembly may be equal to or less than the number of fluid channels in the microdevice. For example, if a microdevice has six fluid channels, a probe assembly with six probes that are insertable within the six fluid channels can be used to substantially simultaneously detect characteristics of the six fluids flowing in the six fluid channels.

In another example, a plurality of optical detectors may be positioned to receive optical signals coming from a plurality of fluids flowing in their respective fluid channels on a microdevice. For example, the plurality of optical detectors may comprise a charge coupled device (CCD) array or a photodiode array. These arrays may be positioned to receive optical signals coming from the fluids flowing in the fluid channels. In some embodiments, radiolabels or fluorescent tags on molecules in fluids flowing in the fluid channels in a microdevice may provide such optical signals.

III. Exemplary methods of manufacture

The microdevices according to embodiments of the invention may be made according to any suitable process. For example, in some embodiments, portions of a body of material may be removed to form a plurality of wall members. In these embodiments, the wall members may be integrally formed with the substrate. Examples of suitable material removal processes include bulk micromachining, sacrificial micromachining, focused ion-beam milling, electrostatic discharge machining, ultrasonic drilling, laser ablation, mechanical milling and thermal molding techniques. Conventional photolithographic and etching processes may be used to etch a body to form a plurality of wall members and fluid channels in the body. Etching processes such as reactive ion etching (RIE) or deep reactive ion etching (DRIE), or wet etching may be used to etch an appropriate body of material. In some embodiments, the wall members and the underlying substrate may be formed by molding. In other embodiments, wall members may be formed on a substrate. For example, wall members may be formed on or bonded to a body to form a plurality of fluid channels. For example, wall members may be formed by electroplating (e.g., high aspect ratio plating).

If desired, after the fluid channels are formed in the microdevice, the surfaces defining the fluid channels may be coated with a material. The material coated on the walls or bottom surfaces defining the channels may be an adhesion layer, coupling agents, or substances that may potentially interact with fluids flowing through the fluid channels.

Any suitable material may be used as to form the substrate and the wall members in the microdevice. The materials used may be organic or inorganic, and may be transparent, translucent, or non-transparent. Materials that can be micromachined or microfabricated are preferred. Suitable micromachinable materials include silicon, glass, plastic and the like. Other suitable materials, and processes for forming a plurality of fluid channels in a microdevice may be found in U.S. Patent Application No. 09/115,397, which is assigned to the same assignee as the present application, and International Application No. PCT/US99/15968. Both of these applications are herein incorporated by reference in their entirety for all purposes.

EXAMPLE

A microdevice having ten fluid channels was fabricated by forming nine wall members in a silicon substrate. The wall members were formed in a silicon substrate using a deep reactive ion etch. Each of the wall members had an opening and the openings in the wall members were aligned to form a slot that passed across the nine wall members. The height of the wall members and the corresponding channel depth was about 200 microns. The width of each of the fluid channels was 110 microns, and the channel pitch was about 150 microns.

Buffered solutions with pH values of 4, 7, and 10 were fed to the different fluid channels in the microdevice. Because the fluid channel volumes were low, the Reynolds number for the solutions in the fluid channels was sufficiently low to maintain laminar flow at reasonable flow rates. With laminar flow, the solutions flowing in the ten fluid channels did not mix in the slot region of the microdevice. The flow rates for the solutions in the fluid channels were set for a maximum value of 500 nanoliters/minute.

The pH values of the different fluids flowing in the ten fluid channels were measured using a scanning probe potentiometer (SPP). The SPP had a probe was insertable into a fluid channel and had a sensitivity of less than 0.01 pH units and a spatial resolution of 10 microns.

The pH of the ten fluids flowing in the ten fluid channels was profiled by measuring the pH in a fluid channel proximate one side of the microdevice. After the pH values in this fluid channel are measured, the probe moves through the slot to the next adjacent fluid channel without removing the pH sensitive area of the probe from the flowing fluids. The pH value of the adjacent fluid channel was then measured. The pH values of the fluids in the remaining eight fluid channels were measured in a similar manner. The travel time between the fluid channels was about 1 second. The measurement time was about 5 seconds per channel.

A plot of sensor potential versus time during the scanning process is shown in FIG. 14. The relative potential difference between each fluid channel correlates closely to the actual pH values of the fluids in the channels (listed above the plot), except for the first and last edge channels. Each of the plateaus in the plot corresponds to a pH measurement of a fluid in a fluid channel. As shown in the plot, the time used to measure the pH values of the ten fluids in the ten fluid channels was less than one minute.

All patents, patent applications, and publications mentioned above are herein incorporated by reference in their entirety. The citation of such documents is not an admission such patents, patent applications, and publications are prior art.

The terms and expressions which have been employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding equivalents of the features shown and described, or portions thereof, it being recognized that various modifications are possible within the scope of the invention claimed. Moreover, any one or more features of any embodiment of the invention may be combined with any one or more other features of any other embodiment of the invention, without departing from the scope of the invention. For example, any feature of the embodiments using multiple detection devices may be used with any feature of the embodiments using wall members with openings without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A microdevice for supporting a flowing fluid, the microdevice comprising:

a substrate; and

a pair of generally parallel, spaced wall members on the substrate, wherein at least one of the wall members includes a pair of structures defining an opening.

2. The microdevice of claim 1 wherein the pair of structures are beveled structures.

3. The microdevice of claim 1 wherein the pair of structures are beveled structures, and wherein each of the beveled structures comprises a pair of inwardly tapering wall surfaces terminating in an apex.

4. The microdevice of claim 3 wherein each of the tapering wall surfaces form an angle of about 2 degrees to about 20 degrees with respect to a side surface of an intermediate portion of the wall member.

5. The microdevice of claim 3 wherein each tapering wall surfaces is curved.

6. The microdevice of claim 1 wherein a distance between the pair of structures is about 50 microns to about 400 microns.

7. The microdevice of claim 1 comprising three or more generally parallel wall members on the substrate.

8. The microdevice of claim 1 wherein the spaced wall members define a fluid channel that contains a fluid with a laminar flow profile.

9. The microdevice of claim 1 further comprising a cover disposed on the wall members.

10. The microdevice of claim 1 wherein each of the wall members include an opening, and wherein the openings in the respective wall members are substantially aligned to form a slot.

11. The microdevice of claim 1 further comprising a slide member, wherein the slide member is disposed on the substrate and is adapted to slide through the opening.

12. An analytical assembly comprising:
the microdevice of claim 1; and
a probe having an end portion that is insertable between the spaced wall members.

13. A microdevice comprising:
a substrate;
a plurality of wall members; and
a plurality of fluid channels, wherein each of the fluid channels is defined by adjacent wall members in the plurality of wall members, wherein each wall member comprises an opening that is formed by opposed beveled structures of the wall member and that communicates the adjacent fluid channels.

14. The microdevice of claim 13 wherein the openings in the respective wall members are substantially aligned to form a slot.

15. The microdevice of claim 13 wherein the openings in each of the wall members are structured to permit fluids having a laminar profile flowing on opposite sides of respective wall members from intermixing.

16. The microdevice of claim 13 further comprising a cover on the wall members and a lid spaced from the cover.

17. A method for detecting a characteristic of a fluid, the method comprising:

(a) inserting a probe into a fluid channel in a microdevice;

(b) detecting a characteristic of a first fluid flowing in the first fluid channel;

(c) moving the probe from the first fluid channel through an opening in one of the wall members defining the first fluid channel and to a second fluid channel adjacent to the first fluid channel; and

(d) detecting a characteristic of a second fluid flowing through the second fluid channel.

18. The method of claim 17 wherein the probe comprises an electrical sensor.

19. The method of claim 17 wherein at least the first fluid contains proteins.

20. The method of claim 17 wherein each of the fluid channels has a width less than about 1000 microns.

21. The method of claim 17 wherein the first and the second fluids comprise a laminar profile.

22. The method of claim 17 wherein (b)-(d) are performed without exposing an end portion of the probe to air.

23. An analytical assembly comprising:

a detection assembly comprising a plurality of detection devices; and

a microdevice comprising a plurality of wall members and a plurality of fluid channels, wherein each of the fluid channels is defined by adjacent wall members in the plurality of wall members.

24. The analytical assembly of claim 23 wherein the plurality of detection devices comprise a plurality of probes.

25. The analytical assembly of claim 23 wherein the plurality of detection devices comprise a plurality of optical detectors.

26. The analytical assembly of claim 23 wherein the detection devices are disposed in the fluid channels in the microdevice.

27. A method for detecting a characteristic of a fluid, the method comprising:

flowing a plurality of different fluids through respective fluid channels in a microdevice, each of the fluid channels in the microdevice being formed by adjacent pairs of wall members; and

detecting characteristics of the plurality of different fluids substantially simultaneously using a plurality of detection devices as the different fluids flow through their respective fluid channels.

28. The method of claim 27 wherein the detection devices comprise a plurality of probes, wherein the plurality of probes is insertable within the plurality of fluid channels.

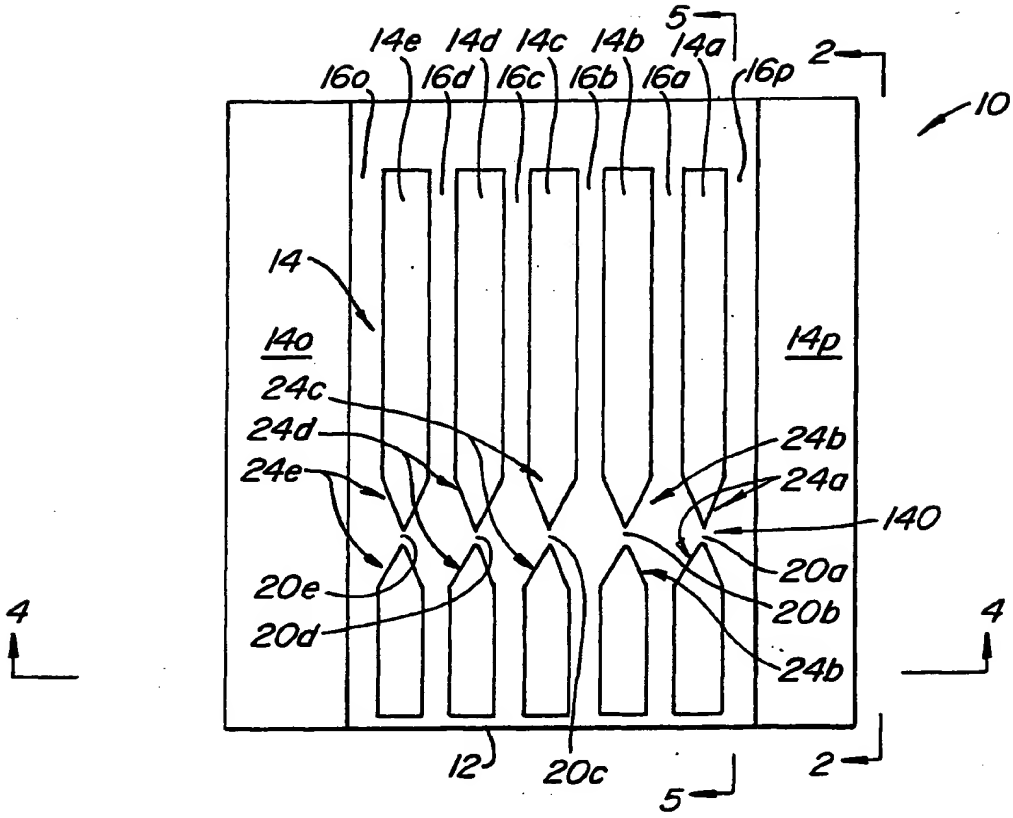

5
14e
14d
14c
14b
14a
2
16o
16d
16c
16b
16a
16p
10
14
14o
14p
24c
24d
24e
24b
24a
140
20a
20e
20b
20d
24b
4
4
12
20c
2
5

FIG. 1.

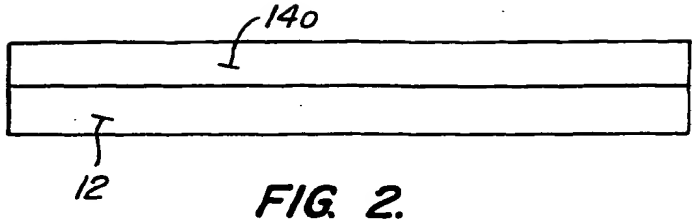


FIG. 2.

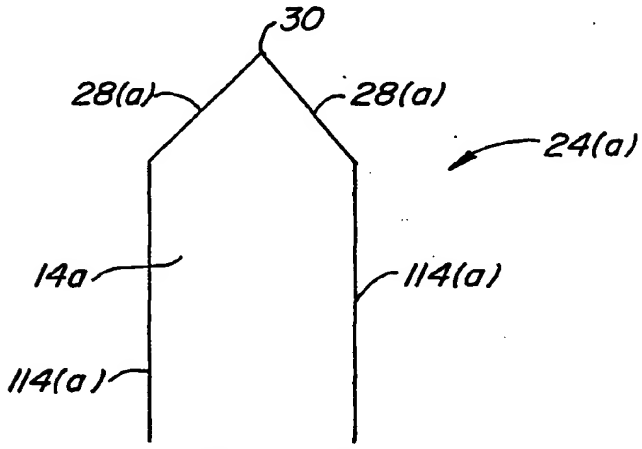


FIG. 3(a).

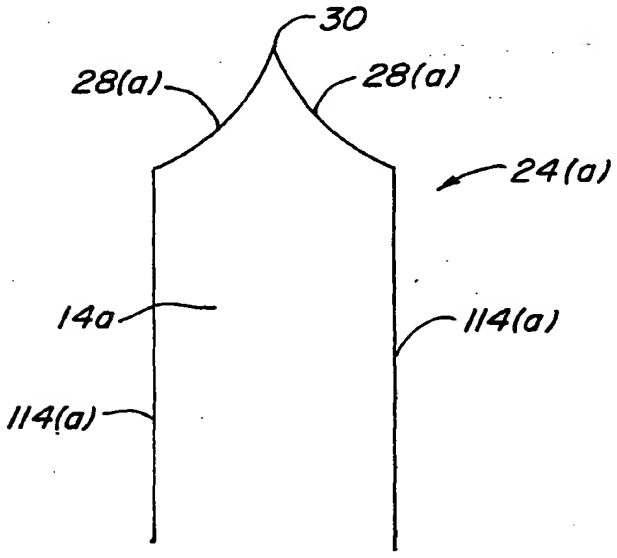


FIG. 3(b).

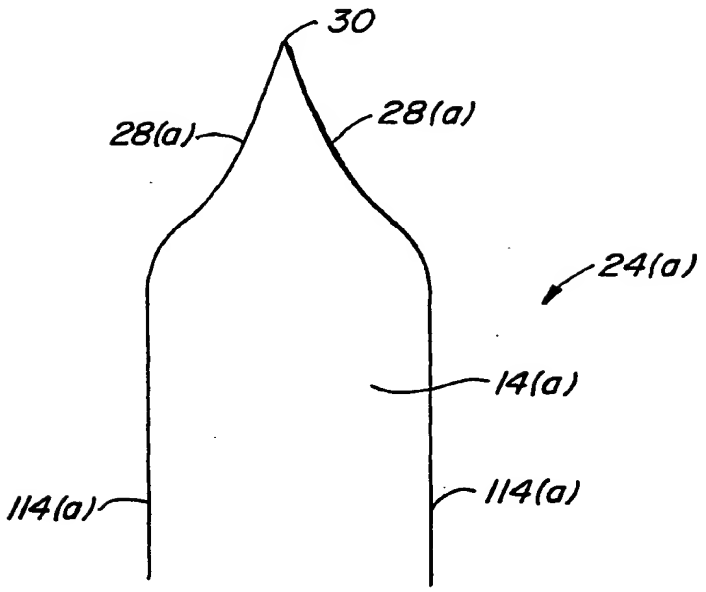

30
28(a)
28(a)
24(a)
14(a)
114(a)
114(a)

FIG. 3(c).

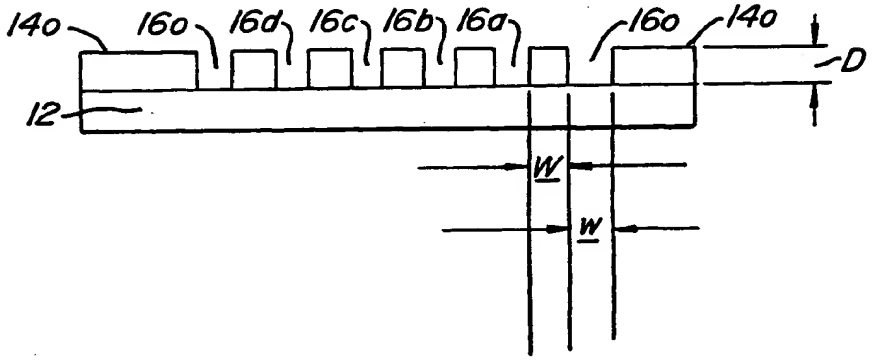

140
160
16d
16c
16b
16a
160
140
D
12
W
W

FIG. 4.

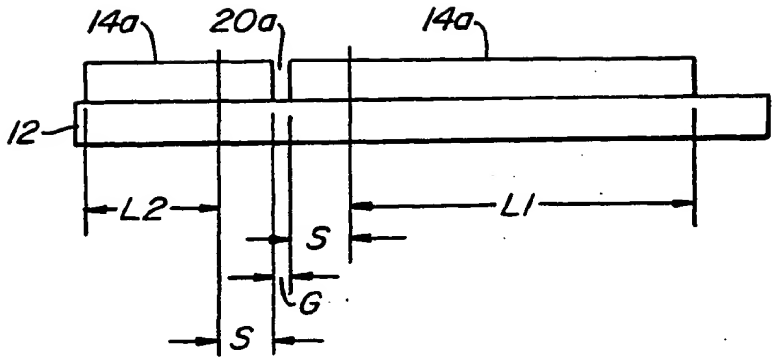


FIG. 5.

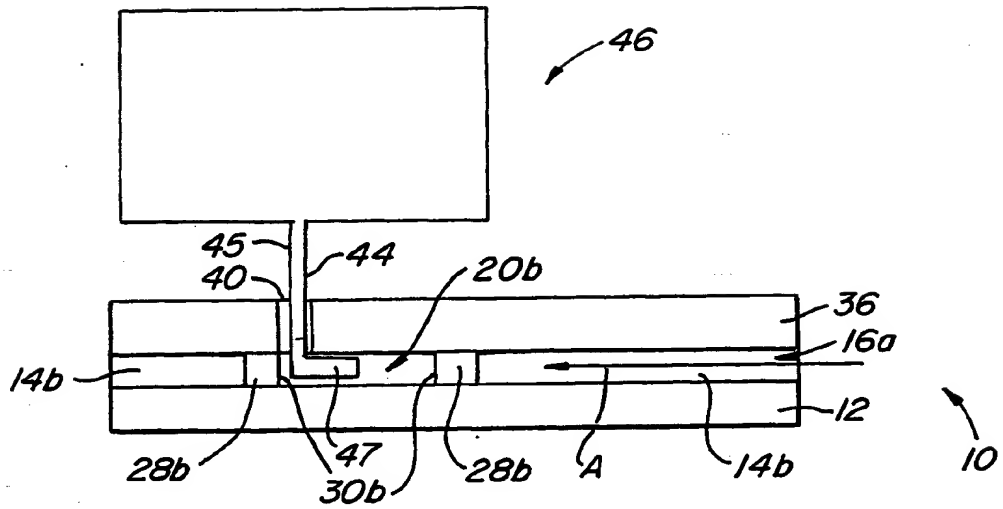
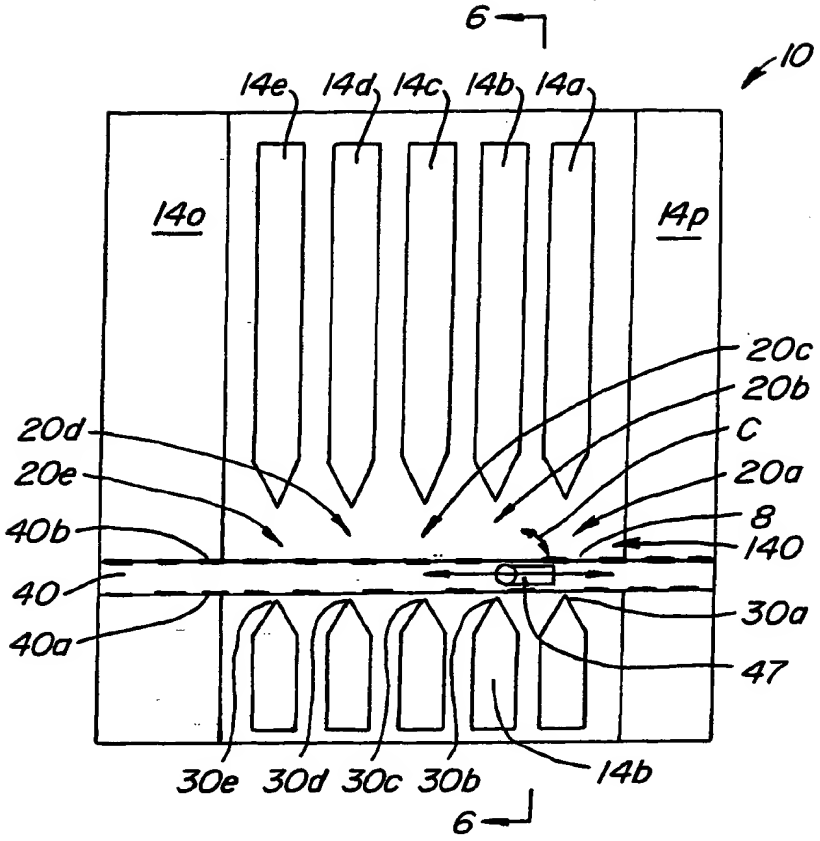


FIG. 6.


6
10
14e
14d
14c
14b
14a
14o
14p
20c
20b
C
20a
8
140
20d
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40
40a
30a
47
30e
30d
30c
30b
14b
6

FIG. 7.

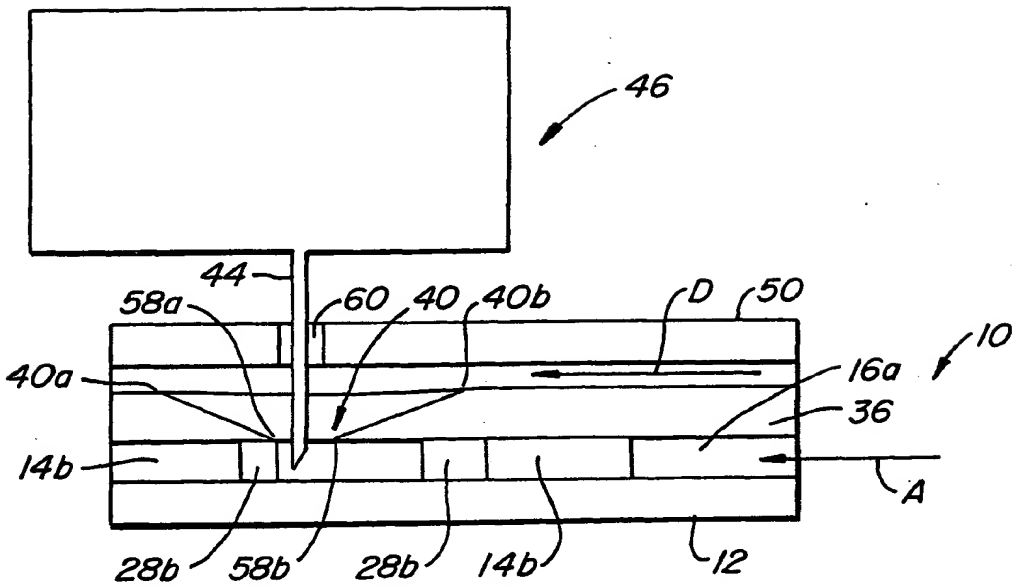

FIG. 8.

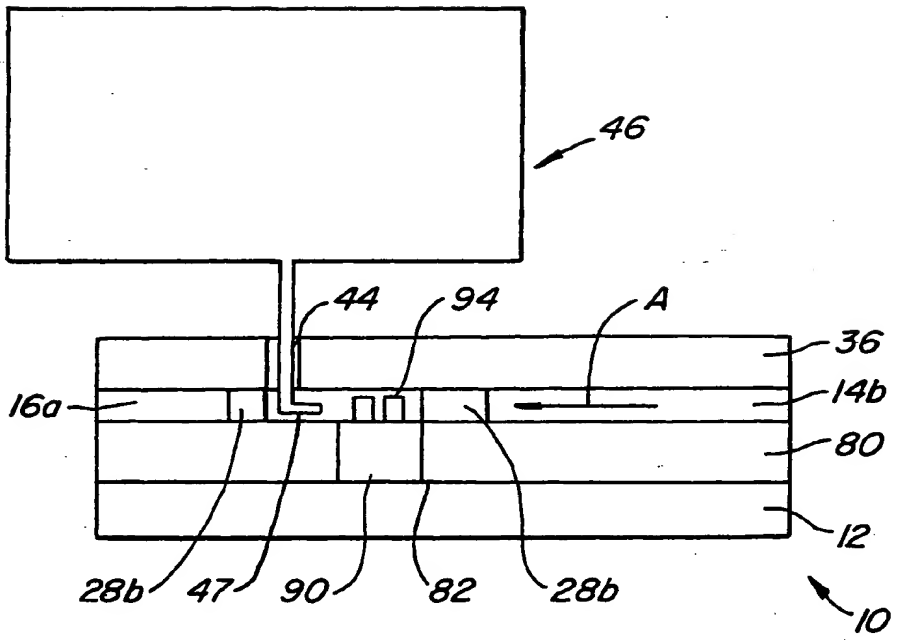

FIG. 9.

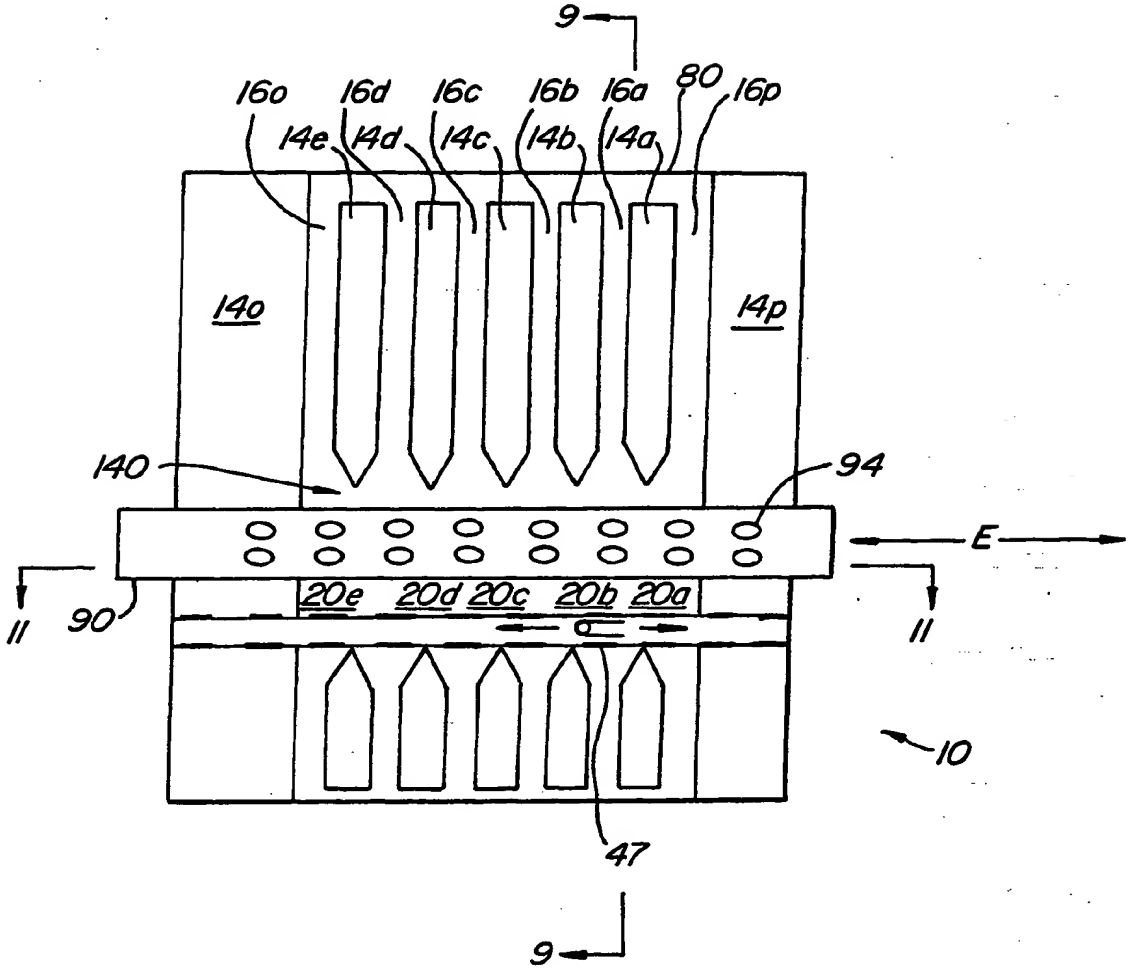
9
16o
16d
16c
16b
16a
80
16p
14e
14d
14c
14b
14a
14o
14p
140
94
E
20e
20d
20c
20b
20a
90
10
47
9

FIG. 10.

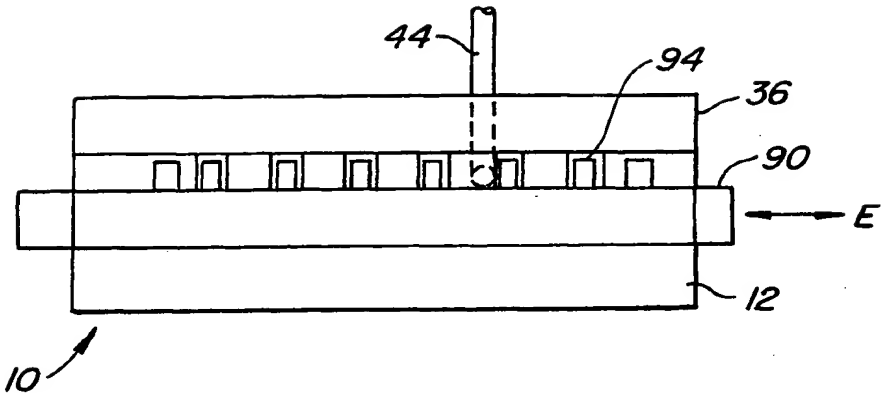

FIG. 11.

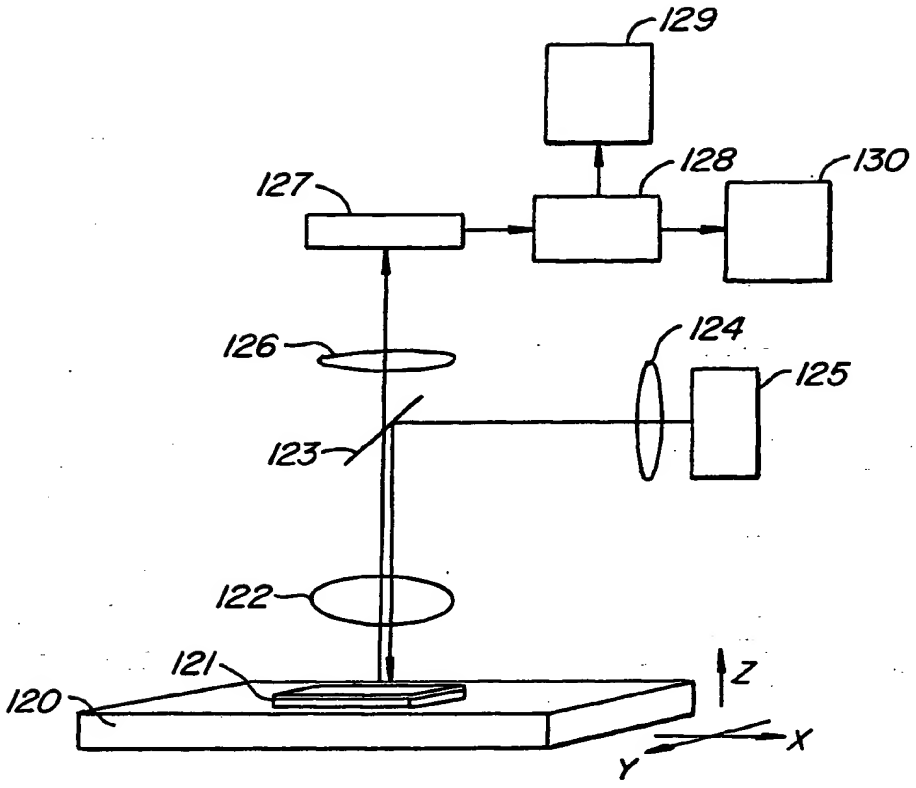

FIG. 12.

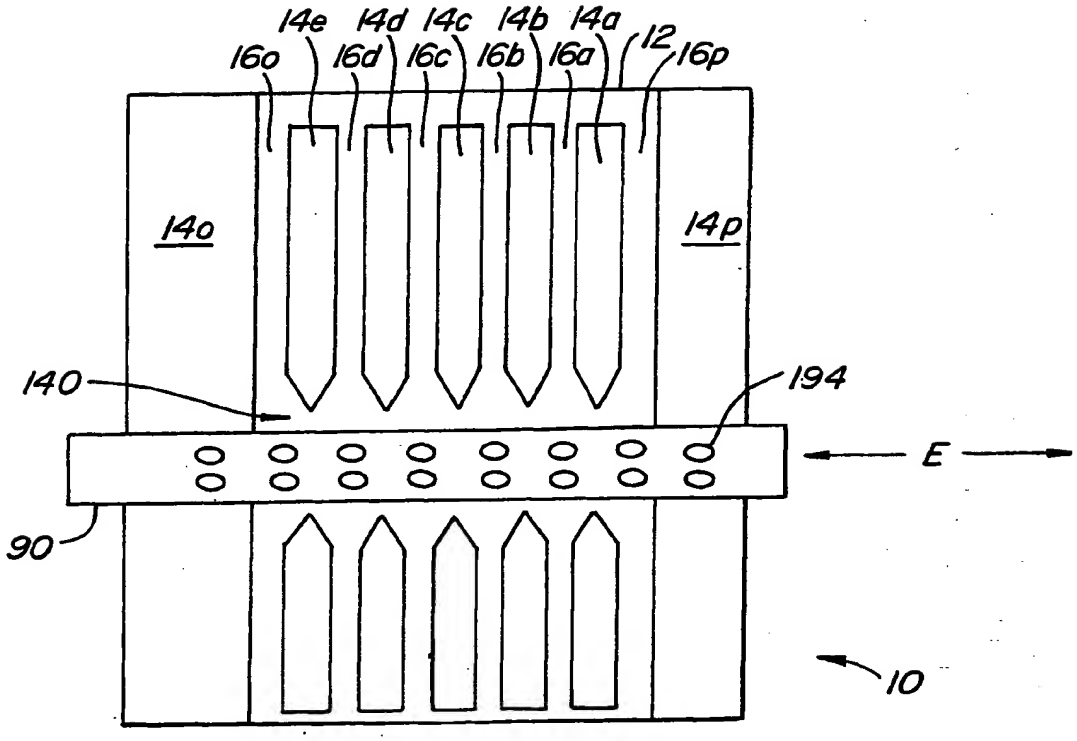
14e
14d
14c
14b
14a
12
16o
16d
16c
16b
16a
16p
14o
14p
140
194
E
90
10

FIG. 13.

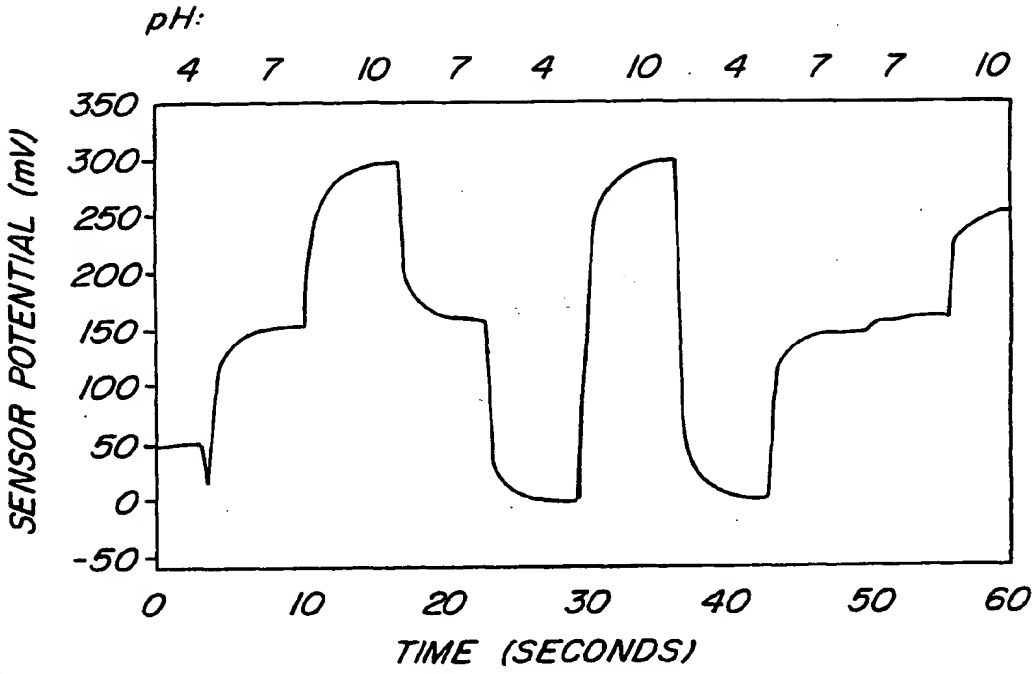
pH:
4 7 10 7 4 10 4 7 7 10
350
300
250
200
150
100
50
0
-50
SENSOR POTENTIAL (mV)
0 10 20 30 40 50 60
TIME (SECONDS)

FIG. 14.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/01198

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : G01N 15/06, 33/00, 33/48
US CL : 422/68.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 422/68.1, 435/287.1, 287.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/04547 A1 (LOCKHEED MARTIN ENERGY SYSTEMS, INC.) 15 February 1996 (15.02.1996).	1-28
Y	US 5,376,252 A (EKSTROM et al.) 27 December 1994 (27.12.1994), see abstract and Figures 1-9.	1-28
A	WOOLLEY et al. Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. Proc. Natl. Acad. Sci., USA. November 1994, Vol. 91, pages 11348-11352.	1-28
A	MANZ et al. Planar chips technology for miniaturization and integration of seaparation techniques into monitoring systems. J. Chromatog. 1992, Vol. 593, pages 253-258.	1-28
A	US 5,635,358 A (WILDING et al.) 03 June 1997 (03.06.1997).	1-28
A	US 5,716,825 A (HANCOCKet al.) 10 February 1998 (10.02.1998).	1-28
Y	US 4,894,146 A (GIDDINGS) 16 January 1990 (16.01.1990), see abstract and Figures 2-7.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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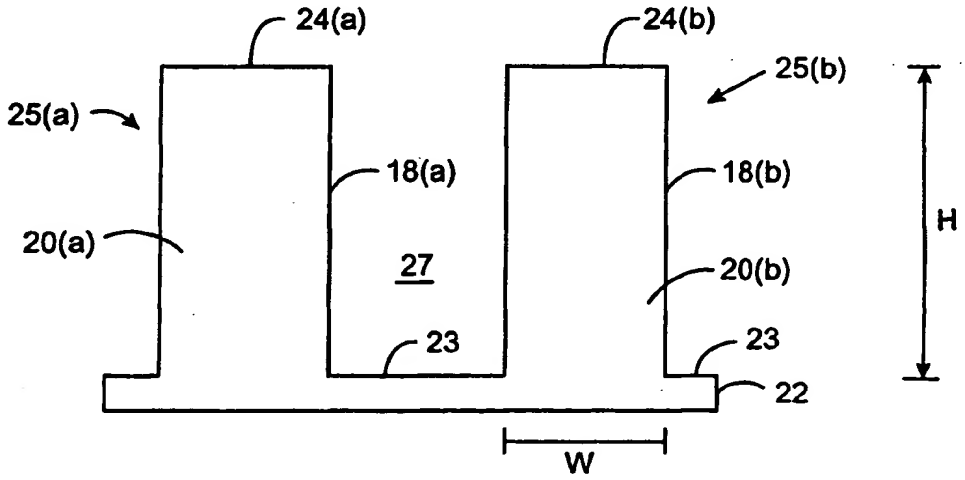
(54) Title: CHIPS HAVING ELEVATED SAMPLE SURFACES



(57) Abstract: A test device is disclosed having a base (22) that is a non-sample surface and sample structures (25 (a,b)) comprising pillars (20 (a,b)).

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CHIPS HAVING ELEVATED SAMPLE SURFACES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application nos. 60/184,381 filed February 23, 2000 and 60/225,999 filed August 16, 2000. This application is also being filed on the same day as U.S. non-provisional application no. _____ entitled "Microfluidic Devices and Methods" by Paul Jedrzejewski et al. (Attorney Docket No. 020144-001510). All of the above provisional and non-provisional patent applications are herein incorporated by reference in their entirety for all purposes and are all assigned to the same assignee as the present application.

BACKGROUND OF THE INVENTION

In the discovery of new drugs, potential drug candidates are generated by identifying chemical compounds with desirable properties. These compounds are sometimes referred to as "lead compounds". Once a lead compound is discovered, variants of the lead compound can be created and evaluated as potential drug candidates.

In order to reduce the time associated with discovering useful drug candidates, high throughput screening (HTS) methods are replacing conventional lead compound identification methods. High throughput screening methods use libraries containing large numbers of potentially desirable compounds. The compounds in the library are numerous and may be made by combinatorial chemistry processes. In a HTS process, the compounds are screened in one or more assays to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or they can be therapeutic.

Conventional HTS processes use multi-well plates having many wells. For example, a typical multi-well plate may have 96 wells. Each of the wells may contain a

different liquid sample to be analyzed. Using a multi-well plate, a number of different liquid samples may be analyzed substantially simultaneously.

FIG. 1 shows a portion of a multi-well plate 10 having a base 17 and a rim 15. The rim 15 extends upward from the base 17 to define a well 16. A micropipette 11 is above the well 16 and dispenses a droplet comprising a liquid sample 13 into the well 16 and onto a sample surface 12. The droplet may have a surface "S". While in the well 16, the rim 15 confines the liquid sample 13 to the sample surface 12 so that it may be analyzed.

It is desirable to reduce the volume of the wells in a multi-well plate to increase the density of the wells on the plate. By doing so, more wells can be present on the plate and more reactions can be analyzed substantially simultaneously. Also, as the volumes of the wells are reduced, the liquid sample volumes are reduced. Reducing the liquid sample volumes reduces the amount of reagents needed in the HTS process. By reducing the amount of reagents used, the costs of the HTS process can be reduced. Also, liquid samples such as samples of biological fluids (e.g., blood) are not always easy to obtain. It is desirable to minimize the amount of sample in an assay in the event that little sample is available.

While it is desirable to increase the density of the wells in a multi-well plate, the density of the wells is limited by the presence of the rims on the wells. The rims could be removed to permit the sample surfaces to be closer together and thus increase the density of the sample surfaces. However, by removing the rims, no physical barrier would be present between adjacent sample surfaces. This increases the likelihood that liquid samples on adjacent sample surfaces could intermix and contaminate each other.

Also, reducing the liquid sample volumes can be problematic. Decreasing the size of assays to volumes smaller than 1 microliter substantially increases the surface-to-volume ratio. Increasing the surface-to-volume ratio increases the likelihood that analytes or capture agents in the liquid sample will be altered, thus affecting any analysis or reaction using the analyte or capture agents. For example, proteins in a liquid sample are prone to denature at liquid/solid and liquid/air interfaces. When a liquid sample containing proteins is formed into a droplet, the droplet can have a high surface area relative to the amount of proteins in the droplet. If the proteins in the liquid sample come into contact with the liquid/air interface, the proteins may denature and become inactive. Furthermore, when the surface-to-volume ratio of a liquid sample increases, the likelihood that the liquid sample will evaporate also increases. Liquids with submicroliter volumes tend to evaporate rapidly when in contact with air. For example, many submicroliter volumes of liquid can evaporate

within seconds to a few minutes. This makes it difficult to analyze or process such liquids. In addition, if the liquid samples contain proteins, the evaporation of the liquid components of the liquid samples can adversely affect (e.g., denature) the proteins.

Embodiments of the invention address these and other problems.

SUMMARY OF THE INVENTION

One embodiment of the invention is directed to a chip comprising: a) a base including a non-sample surface; and b) at least one structure, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive a sample from a dispenser.

Another embodiment of the invention is directed to an assembly adapted to process fluids, the assembly comprising: a) a dispenser comprising a body and at least one fluid channel defined in the body, each fluid channel being adapted to dispense a fluid on one or more of the sample surfaces; and b) a chip comprising (i) a base including a non-sample surface, and (ii) at least one structure, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive the fluid from the dispenser.

Another embodiment of the invention is directed to a method of processing fluids, the method comprising: a) supplying a fluid in a fluid channel in a dispenser; and b) dispensing the fluid on one or more structures on a base of a chip, wherein each structure comprises a pillar and includes a sample surface that is elevated with respect to the non-sample surface.

Another embodiment of the invention is directed to a method of processing fluids, the method comprising: a) supplying a plurality of liquids to respective fluid channels in a dispenser, wherein each of the fluid channels includes a passive valve and wherein the flow of each liquid in each fluid channel stops at the passive valve; b) aligning sample surfaces of a plurality of structures with the plurality of fluid channels, wherein each structure comprises a pillar; and c) contacting the sample surfaces and the liquids in the fluid channels while the sample surfaces are in or are positioned at the ends of the fluid channels.

Another embodiment of the invention is directed to a chip comprising: a) a base including a non-sample surface; and b) a plurality of structures in an array on the base, each structure comprising a pillar and a sample surface that is elevated with respect to the

non-sample surface and is adapted to receive a sample from a dispenser to be processed or analyzed while the sample is on the sample surface.

Another embodiment of the invention is directed to an assembly adapted to process fluids, the assembly comprising: a) a chip comprising: i) a base including a non-sample surface; and ii) a plurality of structures in an array on the base, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive a sample to be processed or analyzed while the sample is on the sample surface; and b) a dispenser including a plurality of fluid channels, each fluid channel including a passive valve, wherein the dispenser dispenses liquid samples on the sample surfaces of the chip.

These and other embodiments are described in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1(a)-1(b) show cross-sections of a micropipette and a micro-well plate.

FIGS. 2(a)-2(b) show cross-sections of chips including pillars.

FIGS. 3 and 4 show cross-sectional views of pillars with affinity structures.

FIG. 5 shows a perspective view of an array of pillars.

FIGS. 6(a) to 6(b) show cross-sections of pillars.

FIGS. 6(c) to 6(h) show perspective views of different types of pillars that may be on a base of a chip.

FIGS. 6(i) to 6(k) show cross-sections of pillars.

FIG. 6(l) shows a cross-sectional view of a chip with pillars having fluid passages passing through them.

FIG. 7 shows a perspective view of a dispenser.

FIG. 8 shows a perspective view of a chip embodiment.

FIG. 9 shows a perspective view of an assembly embodiment.

FIGS. 10-12 shows cross-sectional views of assembly embodiments.

FIG. 13 is a close-up view of a liquid sample on a sample surface of a pillar.

FIG. 14 shows a cross-sectional view of an assembly embodiment.

FIGS. 15 to 16 show cross-sectional views of assembly embodiments.

FIGS. 17(a) to 17(d) show cross-sectional views of an assembly embodiment including a chip with a pillar having a concave side surface.

FIGS. 18 to 23 show cross-sections of various dispenser configurations.

FIG. 24 shows a perspective view of an assembly embodiment.

FIG. 25 shows a perspective cut-away view of a portion of the assembly embodiment shown in FIG. 24.

FIG. 26 shows an exploded view of an assembly embodiment.

FIG. 27 shows a partial perspective, cut-away view of a portion of the assembly embodiment shown in FIG. 26.

FIG. 28 shows an exploded view of an assembly embodiment.

FIGS. 29 to 30 show partial cross-sectional views of the assembly embodiment shown in FIG. 28.

FIG. 31(a) shows a cross-sectional view of an assembly embodiment.

FIG. 31(b) shows a top view of the assembly embodiment shown in FIG. 31(a), with troughs being shown by invisible lines.

It is understood that the above Figures may be simplified or may have disproportionate features in some instances in order to illustrate embodiments of the invention. For example, although FIG. 2(a) shows a chip with two pillars, sample chips according to embodiments of the invention may have any suitable number of pillars. For example, in some embodiments, there may be more than 100 pillars per chip.

DETAILED DESCRIPTION

Embodiments of the invention may be used in any number of different fields. For example, embodiments of the invention may be used in pharmaceutical applications such as proteomic (or the like) studies for target discovery and/or validation as well as in diagnostics in a clinical setting for staging or disease progression. Also, embodiments of the invention may be used in environmental analyses for tracking and the identification of contaminants. In academic research environments, embodiments of the invention may be used in biological or medical research. Embodiments of the invention may also be used with research and clinical microarray systems and devices.

In embodiments of the invention, events such as binding, binding inhibition, reacting, or catalysis between two or more components can be analyzed. For example, the interaction between an analyte in a liquid sample and a capture agent bound to a surface on a pillar may be analyzed using embodiments of the invention. More specifically, interactions

between the following components may be analyzed using embodiments of the invention: antibody/antigen, antibody/hapten, enzyme/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone, receptor/effector, protein/DNA, protein/RNA, repressor/inducer, DNA/DNA and the like.

I. Chips with pillars

One embodiment of the invention is directed to a chip. The chip may comprise a base including a non-sample surface and at least one structure comprising a pillar. The at least one structure is typically in an array on the base of the chip. Each structure includes a sample surface that is elevated with respect to the non-sample surface of the chip. The sample surface of a structure may correspond to the top surface of the pillar. In other embodiments, the sample surface may correspond to an upper surface of a coating on the pillar.

Each sample surface may be adapted to receive a sample to be processed or analyzed while the sample is on the sample surface. The sample may be or include a component that is to be bound, adsorbed, absorbed, reacted, etc. on the sample surface. For example, the sample can be a liquid containing analytes and a liquid medium. In another example, the sample may be the analytes themselves. Because a number of sample surfaces are on each chip, many samples may be processed or analyzed in parallel in embodiments of the invention.

The samples can be in the form of liquids when they contact the sample surfaces. When liquid samples are on the sample surfaces, the liquid samples may be in the form of discrete deposits. Any suitable volume of liquid may be deposited on the sample surfaces. For example, the liquid samples that are deposited on the sample surfaces may be on the order of about 1 microliter or less. In other embodiments, the liquid samples on the sample surfaces may be on the order of about 10 nanoliters or less (e.g., 100 picoliters or less).

In yet other embodiments, discrete deposits of liquids need not be left on the sample surfaces. For example, a liquid containing a capture agent and a liquid medium may contact a sample surface. The capture agent may bind to the sample surface and substantially all of the liquid medium may be removed from the sample surface, leaving only the capture agent at the sample surface. Consequently, in some embodiments of the invention, liquid

media need not be retained on the sample surfaces after liquid from a dispenser contacts the sample surface.

The liquid samples may be derived from biological fluids such as blood and urine. In some embodiments, the biological fluids may include organelles such as cells or molecules such as proteins and nucleic acid strands. When the chip is used to analyze, produce, or process a biological fluid or a biological molecule, the chip may be referred to as a "biochip".

The liquids provided by the dispenser may comprise any suitable liquid media and any suitable components. Suitable components may include analytes, capture agents (e.g., immobilized targets), and reactants. Suitable analytes or capture agents may be organic or inorganic in nature, and may be biological molecules such as polypeptides, DNA, RNA, mRNA, antibodies, antigens, etc. Other suitable analytes may be chemical compounds that may be potential candidate drugs. Reactants may include reagents that can react with other components on the sample surfaces. Suitable reagents may include biological or chemical entities that can process components at the sample surfaces. For instance, a reagent may be an enzyme or other substance that can unfold, cleave, or derivatize the proteins at the sample surface. Suitable liquid media include solutions such as buffers (e.g., acidic, neutral, basic), water, organic solvents, etc.

The elevated sample surfaces upon which the samples are present may have selected properties. In some embodiments, the sample surfaces may be rendered liquiphilic so that the sample surfaces are more likely to receive and retain liquid samples. For example, the sample surfaces may be hydrophilic. Alternatively or additionally, the sample surfaces may have molecules that can bind, adsorb, absorb or react with components in the liquid samples deposited on the sample surfaces. For example, a sample surface may comprise one or more capture agents that may react with an analyte in the liquid sample. In another example, the sample surface may comprise a layer that is capable of receiving and binding the capture agents themselves. Accordingly, in embodiments of the invention, the nature of the sample surface may change as the sample structure changes.

Elevating the sample surfaces with respect to a non-sample surface provides a number of advantages. For example, by elevating the sample surfaces, potential liquid cross-contamination between the liquid samples on adjacent structures is minimized. A liquid sample on a sample surface does not easily flow to an adjacent sample surface, since the sample surfaces are separated by a depression. In some embodiments,

cross-contamination between samples on adjacent sample surfaces is reduced even though rims are not present to confine a liquid sample to a sample surface. Since rims need not be present to confine the samples to their respective sample surfaces, the spacing between adjacent sample surfaces can be reduced, thus increasing the density of the sample surfaces. As a result, more liquid samples may be processed and/or analyzed per chip than in conventional methods. In addition, small liquid sample volumes can be used in embodiments of the invention so that the amount of reagents used is also decreased, thus resulting in lower costs.

In some embodiments, the side or portion of the side surfaces of the structures may be provided with the same selected properties as the sample surface, or different selected properties from the sample surface. For example, the side surfaces of a pillar of a chip may be rendered hydrophobic while the sample surface of the pillar is hydrophilic. The hydrophilic sample surface of a pillar attracts the liquid samples, while the hydrophobic side surfaces of the pillar inhibit the liquid samples from flowing down the sides of the pillars. Accordingly, in some embodiments, a liquid sample may be confined to the sample surface of a pillar without a well rim. Consequently, in embodiments of the invention, cross-contamination between adjacent sample surfaces may be minimized while increasing the density of the sample surfaces.

In an illustrative example of how a chip according to an embodiment of the invention can be used, a first dispenser may deposit a number of liquid samples comprising respectively different proteins on the sample surfaces on a plurality of pillars on the base of the chip. The first dispenser may be a "passive valve" type dispenser. Passive valve type dispensers are described in further detail below. The different proteins, which may be capture agents, may then bind to the different sample surfaces on respectively different pillars. A second dispenser, which may be the same or different than the first dispenser, can then dispense fluids comprising analytes onto the sample surfaces of the pillars. The fluids may remain in contact with the sample surfaces for a predetermined period of time so that analytes in the fluids may have time to interact (e.g., bind, react) with the proteins on the sample surfaces. The predetermined period of time may be greater than about 30 seconds (e.g., greater than about 1 minute). However, the time may vary depending upon the particular interaction taking place. After the predetermined time has elapsed, the sample surfaces of the pillars may be washed and/or exposed to wash or reagent liquids to remove any unbound analytes or reaction products. The wash and/or reagent liquids can address each

pillar independently or jointly, or by exposure to a liquid source through, for example, flooding. The sample surfaces can then be analyzed to determine which, if any, of the analytes in the fluids may have interacted with the bound proteins.

The analysis may take place using any suitable process and may be quantitative or qualitative. The sample surfaces may be analyzed to determine, for example, which analytes bind to the sample surfaces and/or how many analytes are bound to the sample surfaces. In one embodiment, fluorescent tags can be attached to the analytes in the fluids, while the proteins bound to the sample surfaces are free of tags or contain different tags. Binding between the analytes and the bound proteins can be observed or detected by, for example, fluorescence, fluorescence polarization, surface plasmon resonance (SPR), imaging SPR, ellipsometry, or imaging ellipsometry.

In another example of how the chips according to embodiments of the invention may be used, potential drug candidates and a plurality of potential drug candidates can be assayed substantially simultaneously. For instance, synthesized organic compounds may be tested for their ability to act as inhibitors to a family of receptors that are immobilized on different sample surfaces. The synthesized compounds and binding ligands for the receptors may be present in liquid samples that are deposited on the sample surfaces of a chip. Receptors corresponding to the ligands may be immobilized on the sample surfaces. After the liquid samples are deposited on the sample surfaces, a period of time may then pass to allow any potential interactions to occur between the ligands and the receptors. The sample surfaces may then be analyzed to see if the ligands bind to the receptors. If a binding ligand in a liquid sample does not bind to the immobilized receptor, the organic compound dispensed with the ligand may inhibit the interaction between the ligand and the receptor. The organic compound may then be identified as a potential drug candidate.

In another example, liquid samples containing proteins may be deposited on the sample surfaces of the sample structures of a chip. When the sample surfaces receive the liquid samples, they may be within or proximate to the fluid channels of a dispenser. At this point, each fluid channel can serve as a reaction chamber where a reaction can take place. For example, while the sample surfaces of the chip are within or proximate to the fluid channels, various other reagents in liquid samples may be deposited on the previously deposited samples. The reagents can unfold, cleave, or derivatize the proteins in the previously deposited liquid samples. The proteins in the liquid samples may be processed while they are (1) on the sample surfaces, (2) in liquid drops on the sample surfaces, or (3)

while the sample surfaces are in or proximate to the fluid channels. The processed proteins may then be transferred to an analysis device such as a mass spectrometer. In other embodiments, proteins in the deposited liquid samples may, for example, unfold or cleave without subsequently deposited reagents. For example, the proteins in deposited liquid samples may unfold, cleave, or otherwise change if left on the sample surfaces for a predetermined period of time.

Although proteins are mentioned in this example and in other examples, other compounds could serve as a reactant, a catalyst, or an enzyme. A component that is bound to a sample surface may be a counterpart to the reactant, catalyst, or enzyme. It is understood that proteins are cited herein as exemplary samples and components and embodiments of the invention are not limited to the processing or analysis of proteins. In embodiments of the invention, the interaction between any two components may be analyzed.

FIG. 2(a) shows a cross-sectional view of a chip according to an embodiment of the invention. The illustrated chip includes a base **22** and sample structures **25(a), 25(b)** comprising pillars **20(a), 20(b)**. The base **22** and the pillars **20(a), 20(b)** may form an integral structure formed from the same material. Alternatively, the base **22** and the pillars **20(a), 20(b)** may be distinct and may be formed from different materials. Each pillar **20(a), 20(b)** may consist of a single material (e.g., silicon), or may include two or more sections of different materials.

The base **22** of the chip may have any suitable characteristics. For instance, the base **22** of the chip can have any suitable lateral dimensions. For example, in some embodiments, the base **22** can have lateral dimensions less than about 2 square inches. In other embodiments, the base **22** can have lateral dimensions greater than 2 square inches. The non-sample surface of the base **22** may be generally planar. However, in some embodiments, the base **22** may have a non-planar surface. For example, the base **22** may have one or more troughs. The structures containing the sample surfaces and the pillars may be in the trough. Any suitable material may be used in the base **22**. Suitable materials include glass, silicon, or polymeric materials. Preferably, the base **22** comprises a micromachinable material such as silicon.

The pillars **20(a), 20(b)** may be oriented substantially perpendicular with respect to the base **22**. Each of the pillars **20(a), 20(b)** includes a sample surface **24(a), 24(b)** and side surfaces **18(a), 18(b)**. The side surfaces **18(a), 18(b)** of the pillars **20(a), 20(b)** can define respective sample surfaces **24(a), 24(b)** of the pillars **20(a), 20(b)**. The sample

surfaces **24(a), 24(b)** may coincide with the top surfaces of the pillars **20(a), 20(b)** and are elevated with respect to the non-sample surfaces **23** of the chip. The non-sample surfaces **23** and the sample surfaces **24(a), 24(b)** may have the same or different coatings or properties. Adjacent sample surfaces **24(a), 24(b)** are separated by a depression **27** that is formed by adjacent pillars **20(a), 20(b)** and the non-sample surface **23**.

The pillars **20(a), 20(b)** may have any suitable geometry. For example, the cross-sections (e.g., along a radius or width) of the pillars may be circular or polygonal. Each of the pillars **20(a), 20(b)** may also be elongated. While the degree of elongation may vary, in some embodiments, the pillars **20(a), 20(b)** may have an aspect ratio of greater than about 0.25 or more (e.g., 0.25 to 40). In other embodiments, the aspect ratio of the pillars may be about 1.0 or more. The aspect ratio may be defined as the ratio of the height H of each pillar to the smallest width W of the pillar. Preferably, the height of each pillar may be greater than about 1 micron. For example, the height of each pillar may range from about 1 to 10 microns, or from about 10 to about 200 microns. Each pillar may have any suitable width including a width of less than about 0.5 mm (e.g., 100 microns or less).

The liquids (not shown) can be in the form of discrete volumes of liquid and can be present on the sample surfaces **24(a), 24(b)** of the pillars **20(a), 20(b)**, respectively. The liquid samples may be deposited on the sample surfaces **24(a), 24(b)** in any suitable manner and with any suitable dispenser (not shown). The dispenser may include one or more passive valves within the fluid channels in the dispenser. Dispensers with passive valves are described in greater detail below.

The liquid samples may contain components (e.g., analytes, targets, capture agents) that are to be analyzed, reacted, or deposited on the sample surfaces **24(a), 24(b)**. Alternatively or additionally, the liquid samples may contain components that are to be deposited on the surfaces of the pillars **20(a), 20(b)** for subsequent analysis, assaying, or processing. For example, the liquid samples on the pillars **20(a), 20(b)** can comprise proteins. The proteins in the liquid samples may bind to the sample surfaces **24(a), 24(b)**. The proteins on the sample surfaces **24(a), 24(b)** can then be analyzed, processed, and/or subsequently assayed, or used as capture agents for capturing analytes. For example, after binding proteins to the sample surfaces **24(a), 24(b)**, the bound proteins may be used as capture agents. Liquids containing analytes to be assayed against the capture agents may contact the surfaces **24(a), 24(b)**. The sample surfaces may then be analyzed to see if the analytes bind to the protein capture agents.

The liquid samples on the adjacent sample surfaces **24(a), 24(b)** are separated from each other by the depression **27** between the adjacent structures. If, for example, a liquid sample flows off of the sample surface **24(a)**, the liquid sample flows into the depression **27** between the adjacent structures without contacting and contaminating the sample on the adjacent sample surface **24(b)**. To help retain the samples on the sample surfaces **24(a), 24(b)**, the side surfaces **18(a), 18(b)** of the pillars **20(a), 20(b)** may be rendered liquiphobic or may be inherently liquiphobic. For example, the side surfaces **18(a), 18(b)** may be coated with a hydrophobic material or may be inherently hydrophobic. In other embodiments, the side surfaces **18(a), 18(b)** of the pillars may also be coated with a material (e.g., alkane thiols or polyethylene glycol) resistant to analyte binding. The non-sample surface **23** may also be resistant to analyte binding or may be liquiphobic, or may consist partially or fully of the same material as the sample surfaces **24(a), 24(b)**.

In some embodiments, the pillars may have one or more channels that surround, wholly or in part, one or more pillars on the base. Examples of such channels are discussed in U.S. Patent Application No. 09/353,554 which is assigned to the same assignee as the present application and which is herein incorporated by reference in its entirety for all purposes. This U.S. Patent Application also discusses surface treatment processes and compound display processes that can be used in embodiments of the invention.

The top regions of the sample structures **25(a), 25(b)** may include one or more layers of material. For example, FIG. 2(b) shows a cross-sectional view of a chip with pillars **20(a), 20(b)** having a first layer **26** and a second layer **29** on the top surfaces **19(a), 19(b)** of the pillars **20(a), 20(b)**. In this example, the sample surfaces **24(a), 24(b)** of the structures **25(a), 25(b)** may correspond to the upper surface of the second layer **29**. In some embodiments, the top regions of the structures **25(a), 25(b)** may be inherently hydrophilic or rendered hydrophilic. As explained in further detail below, hydrophilic surfaces are less likely to adversely affect proteins that may be at the top regions of the structures **25(a), 25(b)**.

The first and the second layers **26, 29** may comprise any suitable material having any suitable thickness. The first and the second layers **26, 29** can comprise inorganic materials and may comprise at least one of a metal or an oxide such as a metal oxide. The selection of the material used in, for example, the second layer **29** (or for any other layer or at the top of the pillar) may depend on the molecules that are to be bound to the second layer **29**. For example, metals such as platinum, gold, and silver may be suitable for use with linking agents such as sulfur containing linking agents (e.g., alkanethiols or disulfide linking agents),

while oxides such as silicon oxide or titanium oxide are suitable for use with linking agents such as silane-based linking agents. The linking agents can be used to couple entities such as capture agents to the pillars.

Illustratively, the first layer **26** may comprise an adhesion metal such as titanium and may be less than about 5 nanometers thick. The second layer **29** may comprise a noble metal such as gold and may be about 100 to about 200 nanometers thick. In another embodiment, the first layer **26** may comprise an oxide such as silicon oxide or titanium oxide, while the second layer **29** may comprise a metal (e.g., noble metals) such as gold or silver. Although the example shown in FIG. 2(b) shows two layers of material on the top surfaces **19(a), 19(b)** of the pillars **20(a), 20(b)**, the top surfaces **19(a), 19(b)** may have more or less then two layers (e.g., one layer) on them. Moreover, although the first and the second layers **26, 29** are described as having specific materials, it is understood that the first and the second layers **26, 29** may have any suitable combination of materials.

The layers on the pillars may be deposited using any suitable process. For example, the previously described layers may be deposited using processes such as electron beam or thermal beam evaporation, chemical vapor deposition, sputtering, or any other technique known in the art.

In embodiments of the invention, an affinity structure may be on a pillar, alone or in combination with other layers. For example, the affinity structure may be on an oxide or metal layer on a pillar or may be on a pillar without an intervening layer. Preferably, the affinity structure comprises organic materials. In some embodiments, the affinity structure may consist of a single layer comprising molecules that are capable of binding to specific analytes (e.g., proteins). For instance, the affinity structure may comprise a single layer of capture agents that are bound to the surface of, for example, a metal or oxide layer on a pillar. The capture agents may comprise, for example, antibodies, antibody fragments, polypeptides, receptors, DNA strands, fragments, RNA strands or fragments, aptamers, etc. The capture agents can bind to components in a liquid medium through a covalent or a non-covalent mechanism. The affinity structure (and the elements of the affinity structure) can be used to increase the spacing between a top surface (e.g., a silicon surface) of a pillar and a protein that is attached to the top surface of the pillar. The spacing can decrease the likelihood that the attached protein might become deactivated by, for example contacting a solid surface of the sample structure.

In other embodiments, the affinity structure may comprise an organic thin film, affinity tags, adaptor molecules, and capture agents, alone or in any suitable combination. When any of these are used together, the organic thin film, affinity tags, adaptor molecules, and the capture agents may be present in two or more sublayers in the affinity structure. For example, the affinity structure may include three sublayers, each sublayer respectively comprising an organic thin film, affinity tags, and adaptor molecules.

The organic thin film, affinity tags, and adaptor molecules may have any suitable characteristics. An "organic thin film" is a normally a thin layer of organic molecules that is typically less than about 20 nanometers thick. Preferably, the organic thin film is in the form of a monolayer. A "monolayer" is a layer of molecules that is one molecule thick. In some embodiments, the molecules in the monolayer may be oriented perpendicular, or at an angle with respect to the surface to which the molecules are bound. The monolayer may resemble a "carpet" of molecules. The molecules in the monolayer may be relatively densely packed so that proteins that are above the monolayer do not contact the layer underneath the monolayer. Packing the molecules together in a monolayer decreases the likelihood that proteins above the monolayer will pass through the monolayer and contact a solid surface of the sample structure. An "affinity tag" is a functional moiety capable of directly or indirectly immobilizing a component such as a protein. The affinity tag may include a polypeptide that has a functional group that reacts with another functional group on a molecule in the organic thin film. Suitable affinity tags include avidin and streptavidin. An "adaptor" may be an entity that directly or indirectly links an affinity tag to a pillar. In some embodiments, an adaptor may provide an indirect or direct link between an affinity tag and a capture agent. Alternatively or additionally, the adaptor may provide an indirect or direct link between the pillar and, an affinity tag or a capture agent. The capture agent is preferably capable of capturing a protein from a liquid sample. In yet other embodiments, an adaptor may bind directly to a pillar or a layer on a pillar, and may be capable of binding to a component such as an analyte in a liquid sample. An example of a suitable adaptor is biotin. Other examples of organic thin films, affinity tags, adaptors, and capture agents are described in U.S. Patent Application nos. 09/115,455, 09/353,215, and 09/353,555, which are herein incorporated by reference in their entirety for all purposes, and are assigned to the same assignee as the present application. These U.S. Patent Applications describe various layered structures that can be on the pillars in embodiments of the invention.

The use of an affinity tag provides several advantages. For example, an affinity tag can confer enhanced binding or reaction of the protein with an underlying organic thin film. Proteins, for instance, can be immobilized in a manner that does not require harsh reaction conditions that are adverse to protein stability or function.

The affinity structures and their sublayers may be formed using any suitable process including, for example, chemisorption, physisorption or chemoselective ligation processes. The materials of the sublayers may be bound to the other sublayer materials, the pillars, or layers on the pillars by a covalent or a non-covalent bonding mechanism.

Examples of chip structures having affinity structures on the pillars are shown in FIGS. 3 and 4. FIG. 3 shows a cross-sectional view of a sample structure having an elevated sample surface. The sample structure includes a pillar **60**. An interlayer **61** including an oxide such as silicon oxide is at the top surface of the pillar **60**. The interlayer **61** may be used to bind the coating layer **62** to the pillar **60**. The coating layer **62** may include another oxide such as titanium oxide. An affinity structure **69** is on the coating layer **62**. The affinity structure **69** may include a monolayer **64** with organic molecules such as polylysine or polyethylene glycol. In some embodiments, the molecules in the monolayer **64** are linear molecules that may be oriented generally perpendicular to, or at an angle with, the surface the coating layer **62**. Each of the organic molecules in the monolayer **64** may have functional groups at both ends to allow the ends of the molecules to bind to other molecules. A set of molecules including a first adaptor molecule **65** such as biotin, an affinity tag **66** such as avidin or streptavidan, a second adaptor molecule **67** such as biotin, and a capture agent **68** such as an antibody are linked together. The set of molecules is bound to the monolayer **64**. In this example, the capture agent **68** is adapted to receive and capture an analyte in a liquid sample that is on the pillar **60**. For simplicity of illustration, only one set of molecules is shown in FIG. 3. However, it is understood that in embodiments of the invention, many such sets of molecules may be present on the monolayer **64**.

The embodiment shown in FIG. 3 has an affinity structure that has a number of sublayers. The affinity structures used in other embodiments of the invention may include more or less sublayers. For example, FIG. 4 shows a cross-sectional view of another sample structure having an affinity structure with fewer sublayers. The structure shown in FIG. 4 includes a pillar **70**. An interlayer **71** including a material such as silicon dioxide is at the top surface of the pillar **70**. A coating layer **72** including, for example, a metal oxide (e.g., titanium oxide) may be on the interlayer **71**. An affinity structure **78** may be on the coating

layer 72. The affinity structure 78 may include a monolayer 73, an affinity tag 74, and an adaptor molecule 75. The affinity tag 74 may be on the monolayer 73 and may couple the adaptor molecule 75 to the monolayer 73. The adaptor molecule 75 may in turn bind an analyte 76 such as a protein to the affinity tag 74.

The affinity structure components separate the sample surface from the top surface of the pillar. As noted above, proteins may deactivate when they come into contact with certain solid surfaces. The affinity structure may serve as a barrier between the pillar and any components in a liquid sample that are to be captured. This reduces the possibility that the top surface of the pillar may deactivate proteins in a liquid sample on the pillar. As shown in FIGS. 3 and 4, for example, the bound analyte 76 and the bound capture agent 68 are not in likely to contact a solid surface (e.g., the solid surfaces of the coating layers 62, 72). Consequently, the presence of the affinity structure 69, 78 decreases the likelihood that contact sensitive molecules such as proteins will be adversely affected by contact with a solid surface. To further reduce this possibility, the materials of the affinity structure may contain materials that are less likely to inactivate proteins.

The pillars may be present in an array on a base of the chip. An example of an array of pillars is shown in FIG. 5. The pillar array may be regular or irregular. For example, the array may have even rows of pillars forming a regular array of pillars. The density of the pillars in the array may vary. For example, the density of the pillars may be about 25 pillars per square centimeter or greater (e.g., 10,000 or 100,000 per cm^2 or greater). Although the chips may have any suitable number of pillars, in some embodiments, the number of pillars per chip may be greater than 10, 100, or 1000. The pillar pitch (i.e., the center-to-center distance between adjacent pillars) may be 500 microns or less (e.g., 150 microns).

FIGS. 6(a)-6(b) show cross-sections of some pillar embodiments. FIG. 6(a) shows a pillar 24 that is integrally formed with respect to an underlying base 22. In such embodiments, the base 22 may consist of the same material as the pillar 24. FIG. 6(b) shows a pillar 24 that is on a base 22. The pillar 24 may include, for example, a porous material such as a hydrogel material. In embodiments of the invention, all, part, or parts of the pillar may be similarly or differently porous (e.g., may have the same or different degree of porosity). For instance, different strata within a pillar may be porous and can have different properties. By using a porous material, liquid samples can pass into the porous material, and the pillar 24 can hold more liquid sample than would be possible if the pillar 24 was non-porous. Consequently, more liquid sample can be present in a porous pillar than on a

non-porous pillar of similar cross-sectional dimensions. If the liquid sample contains a fluorescent material, for example, more fluorescent material would be retained by the pillar than would be the case with a non-porous pillar. A higher quality signal (e.g., a stronger signal) may be produced as a result of the increased amount of fluorescent material in the porous pillar as compared with a non-porous pillar that may only have fluorescent material on the top surface of the pillar.

Other suitable pillar shapes are shown in FIGS. 6(c) to 6(k). The embodiment shown in FIG. 6(i) includes a depression at the top portion of the pillar. In this embodiment, the sample surface may lie below the topmost portion of the pillar.

FIGS. 6(j) and 6(k) show pillars with concave portions. In the embodiment shown in FIG. 6(j), each of the pillars **410, 420** has two non-concave portions **400, 402**, one portion **400** near the top and one portion **402** near the bottom. In this example, the side surface of each non-concave portion **400, 402** is substantially perpendicular to the top surface **406** of the pillar. A concave portion **404** lies between the two non-concave portions **400, 402**. Each pillar includes abrupt changes in geometry where the concave portion **404** begins and ends. The concave portions **400, 402** may be formed using, for example, a reactive ion etch process. FIG. 6(k) shows a pillar with a concave side surface that begins at the top surface of the pillar and ends at the bottom surface of the pillar.

Using pillars with concave portions and abrupt structural changes can be advantageous. For example, by providing concave portions to the pillars, more empty space is provided in the regions between adjacent pillars. For example, referring to FIG. 6(j), the volume V between the adjacent pillars **410, 420** can be used to contain any liquid sample that may flow off of the sample surfaces of the pillars **410, 420**. The volume V between adjacent pillars **410, 420** with concave portions is greater than the volume between adjacent pillars having substantially parallel side surfaces (compare, e.g., the pillars shown in FIG. 6(a)). Consequently, more space is provided to contain any liquids that may inadvertently flow off of the sample surfaces of the pillars. Moreover, the upper non-concave portion **400** of the pillar **410** shown in FIG. 6(j) has two structurally distinct edges E1, E2. As will be explained in further detail below, when pillars with abrupt structural changes (e.g., in FIG. 6(j)) are used, these structural changes can form two passive valves when used in conjunction with a dispenser with a cooperatively structured fluid channel. The two passive valves help to prevent a liquid sample from flowing down the sides of the pillars **410, 420**. Furthermore, if a liquid sample flows off of the sample surface on the pillar, the concave surface of the pillar

can provide a path for the liquid sample to flow inwardly and away from an adjacent sample surface. This also reduces the likelihood of potential liquid cross-contamination between adjacent sample surfaces.

In some embodiments, fluid passages may also be provided in the pillars of the chip. For example, FIG. 6(l) shows pillars **299** on a base **290**. A fluid passage **294** extends through both the base **290** and the pillars **299**. A fluid **292** such as a gas may pass through the fluid passages **294** toward the sample surfaces on the pillars **299** to remove substances from the sample surfaces. A cover chip **291** with corresponding apertures may be placed over the fluid passages **294** in the pillar **299** so that the apertures are over the sample surfaces. Gas may flow through the fluid passages **294** to carry processed samples **295** on the upper surfaces of the pillars **299** to an analytical device such as a mass spectrometer.

In a typical process of using the assembly shown in FIG. 6(l), liquids from a dispenser (not shown) may contact the sample surfaces on the pillars of a sample chip. The liquids may process substances on the sample surfaces on the pillars. For example, the liquids may comprise reagents that process proteins on the sample surfaces. After processing, the chip is separated from the dispenser, and the cover chip **291** is placed on the sample chip with the pillars **299**. The apertures of the cover chip **291** are respectively over the sample surfaces, and gas flows through fluid passages **294** that extend through the pillars **299**. The gas removes the processed substances from the sample surfaces and carries the processed substances through the apertures in the cover chip **291** and to an analysis device such as a mass spectrometer.

The sample chip shown in FIG. 6(l) can be used in other ways. For example, in other embodiments of the invention, liquids may also pass upwards through the fluid passages **294** and deposit on the sample surfaces of the sample chip (i.e., on the pillars). In yet other embodiments, the fluid passages **294** can be used to keep components at the sample surfaces hydrated. Hydrating gases or liquids (e.g., water) can pass through the fluid passages **294** to keep any components on the sample surfaces hydrated. For example, by keeping proteins on the sample surfaces hydrated, the proteins are less likely to denature. In some embodiments, the fluid passages **294** may be coupled to a sub-strata porous region of the pillar, useful, for example, to act as a liquid reservoir to supply liquid to the sample surface.

The pillars of the chip may be fabricated in any suitable manner and using any suitable material. For example, an embossing, etching or a molding process may be used to

form the pillars on the base of the chip. For example, a silicon substrate can be patterned with photoresist where the top surfaces of the pillars are to be formed. An etching process such as a deep reactive ion etch may then be performed to etch deep profiles in the silicon substrate and to form a plurality of pillars. Side profiles of the pillars may be modified by adjusting process parameters such as the ion energy used in a reactive ion etch process. If desired, the side surfaces of the formed pillars may be coated with material such as a hydrophobic material while the top surfaces of the pillars are covered with photoresist. After coating, the photoresist may be removed from the top surfaces of the pillars. Processes for fabricating pillars are well known in the semiconductor and MEMS (microelectromechanical systems) industries.

II. Assemblies

Other embodiments of the invention are directed to fluid assemblies. The fluid assemblies according to embodiments of the invention may include a sample chip and a dispenser that can dispense one or more fluids on the sample surfaces of the chip. In some embodiments, a plurality of liquids may be supplied to the fluid channels in a dispenser. The liquids supplied to the different fluid channels may be the same or different and may contain the same or different components. For example, each of the liquids in respective fluid channels may include different analytes to be assayed. In another example, the liquids in respective fluid channels may contain different capture agents to be coupled to the pillars of the sample chip. The dispenser may provide liquids to the sample surfaces in parallel.

The chips used in the assemblies may be the same or different than the previously described chips. For example, the chips in the assemblies may include structures having elevated sample surfaces and pillars.

The dispenser may have any suitable characteristics, and can be positioned above the sample chip when liquids are dispensed onto the sample chip. Pressure may be applied to the liquids to dispense the liquids. To control liquid flow, the dispenser may include passive or active valves.

Active liquid valves are well known in the art. These valves control the flow or location of a liquid by actively changing a physical parameter. Some examples follow: 1) heat or light change the liquiphilic properties of a polymer which may be

used to control the location of a liquid 2) electric potential can be used to induce an electrokinetic flow 3) MEMS structures can be used to block or unblock a liquid channel 4) the movement of magnetic particles or features in a channel can influence the liquid behavior.

In some embodiments, the dispensers have at least one passive valve per fluid channel. Preferably, the dispenser includes a plurality of nozzles. The plurality of nozzles can provide different liquids containing different components to different sample surfaces of the pillars substantially simultaneously. For instance, if there is an array of one hundred sample surfaces on a chip, then a dispenser may have one hundred sample nozzles that are arranged in a pattern similar to the array of sample surfaces. In other embodiments, the dispenser may have one or more nozzles that provide liquids on different sample surfaces in series. Examples of dispensers that can be used in embodiments of the invention include ring-pin dispensers, micropipettes, capillary dispensers, ink-jet dispensers, hydrogel stampers, and dispensers comprising passive valves. In some embodiments, the dispensers may be in the form of a chip with a plurality of fluid channels. In these embodiments, each of the fluid channels can have an end that terminates at a bottom face of the dispenser chip. The dimensions of the fluid channels in the dispenser may vary. For example, a cross-sectional dimension of a fluid channel in the dispenser may be between about 1.0 to about 500 microns (e.g., about 1.0 to about 100 microns).

The dispensers used in embodiments of the invention may be made using any suitable process know in the art. For example, the dispenser may be made, for example, by a 3-D stereo lithography, mechanical drilling, ion etching, or a reactive ion etching process.

In some assembly embodiments, the sample structures of the chip may be cooperatively structured to fit into fluid channels in a dispenser. The sample structures and their corresponding sample surfaces may be aligned with the fluid channels. After aligning, the sample surfaces may be positioned in the fluid channels or at the ends of the fluid channels. Fluids in the fluid channels may then contact the sample surfaces of the structures. For example, pressure (e.g., caused by pneumatic forces, electrophoretic or electrowetting forces) may be applied to a liquid in a fluid channel so that the liquid flows and contacts the sample surface in the fluid channel. In other embodiments, the distance between the sample surface and the liquid in a fluid channel may decrease until they contact each other. The chip and/or the dispenser may move toward each other to decrease the spacing between the sample

surface and the liquid in the fluid channel. In these embodiments, pressure may or may not be applied to the liquid in the fluid channel.

The fluid channels in the dispenser may serve as reaction chambers (or interaction chambers) that can house respectively different interactions such as reactions or binding events. Each sample surface and the walls of a corresponding fluid channel may form a reaction chamber. In a typical assembly, each individual reaction chamber may house a different event (e.g., a different reaction or binding event). In other embodiments, the different reaction chambers may house the same types of events.

Illustratively, a dispenser may provide liquids to the sample surfaces of the chip structures. The liquids may contain molecules that may or may not interact with molecules bound to the sample surfaces of the chip. First, the sample structures containing the sample surfaces may be aligned with the fluid channels. After aligning, the sample surfaces may be inserted into or positioned proximate to the fluid channels. While the sample surfaces are in or proximate to the fluid channels, the liquids in the fluid channels of the dispenser flow and contact the sample surfaces. This allows the molecules bound to the sample surfaces and the molecules in the liquids to react or interact with each other in a nearly closed environment. The interactions or reactions can take place minimizing the exposure of the liquid samples on the sample surfaces to a gaseous environment such as air. Consequently, the likelihood that the liquid samples will evaporate is reduced. After a predetermined time has elapsed, the sample surfaces may be withdrawn from the fluid channels, and/or the chip and the dispenser may be separated from each other. The sample surfaces of the chip can then be rinsed. Products of the reactions or interactions may remain on the sample surfaces. The products at the sample surfaces may then be analyzed to determine, for example, if a reaction has taken place. Alternatively or additionally, the products on the sample surfaces may be further processed or may be separated from the chip and may be transferred downstream of the sample surfaces for further processing or analysis. In other embodiments, the products at the sample surface may be capture agents that can be used to capture analytes in liquids.

Embodiments of the invention may be used to transfer liquids containing capture agents, analytes, etc. to sample surfaces of a chip without forming droplets. For example, a liquid need not pass through a gaseous medium (e.g., air) when it is transferred from a dispenser to the chip. This minimizes the creation of liquid volumes with large surface-to-volume ratios. In embodiments of the invention, small volumes of liquids may be

transferred to a chip and processed on the chip while minimizing alterations (e.g., protein denaturing) of components in the transferred liquids.

Some assembly embodiments may be described with reference to FIGS. 7 to 9. FIG. 7 shows a dispenser **110** and FIG. 8 shows a chip **105**. The chip **105** includes a plurality of pillars **101** on a base **105a**. Each pillar **101** has a top sample surface **103** and a side surface **104**. The sample surface **103** is elevated with respect to a non-sample surface of the base **105a**.

The dispenser **110** includes a body **111** having at least one fluid channel **112** defined in the body **111**. In this example, the fluid channels **112** are substantially vertical. As noted above, the fluid channels **112** may define reaction chambers that can house chemical or biological reactions or interactions. At least a portion of the fluid channels **112** may be oriented in a z direction with respect to an x-y plane formed by the body **111** of the dispenser **110**. In this example, the fluid channels **112** illustrated in FIG. 7 are vertical and have one end terminating at an upper surface of the body **111** and the other end terminating at a lower surface of the body **111**.

In other dispenser embodiments, the fluid channels **112** may have horizontal and vertical portions. For example, one end of a fluid channel may originate at an upper surface of the body and may pass horizontally across the upper surface of the body. At some predetermined point on the body, the orientation of the fluid channel changes from a horizontal orientation to a vertical orientation and terminates at a lower surface of the body of the dispenser. Moreover, although the number of fluid channels **112** in the dispenser is shown to be equal to the number of pillars **101** in the assembly shown in FIGS. 7 and 8, the number of fluid channels and the number of pillars of a chip may be different in other embodiments.

The walls defining the fluid channels **112**, as well as a bottom surface **113** of the dispenser **110** may be coated with various materials that influence the behavior of the liquid in the fluid channels **112** (e.g., wetting). For instance, the fluid channel walls may be coated with materials that increase or decrease the interaction between fluid channel walls and the liquids in the fluid channels. For example, the walls defining the fluid channels **112** may be coated with a hydrophilic material. Proteins, for example, are less likely to denature if they come in contact with a hydrophilic surface than with a non-hydrophilic surface.

The fluid channels **112** in the dispenser **110** may be cooperatively structured to receive the pillars **101**. For example, as shown in FIG. 8, the pillars **101** of the chip **105** may

be insertable into the fluid channels **112** in the body of the dispenser **110**. In this regard, the axial cross-sectional area of each of the fluid channels **112** in the dispenser **110** may be greater than the axial cross-sectional area of the pillars **101**. When the pillars **101** are inserted into the fluid channels **112** in the dispenser **110**, the sample surfaces **103** of the pillars **101** may be within respective fluid channels **112**. The volumes defined by the fluid channels **112** and the top surfaces **103** of the pillars **101** may be reaction chambers where reactions can occur.

The chip **105** and the dispenser **110** may each have one or more alignment members so that they can be aligned with each other and the pillars can be aligned with the fluid channels. The alignment members may be alignment marks or alignment structures. Typical alignment structures may be, for example, a pin and a corresponding hole. For instance, the edges of the chip **105** may have one or more pins (not shown) that are longer than the pillars **101**. These pins may be inserted into corresponding holes (not shown) at the edges of the dispenser **110** to align the chip **105** and the dispenser **110** and consequently align the pillars **101** with the fluid channels **112**. The alignment members may be optical, mechanical, or magnetic. For example, in some embodiments, the alignment members may be high aspect ratio linear channels which permit light passage when, for example, the chip and the dispenser are operatively aligned. Alternatively, a magnetic region may induce a signal in a detector once, for example, the chip and the dispenser are operatively aligned

The assembly embodiments may be used to perform assays. Illustratively, biological molecules such as proteins may be bound to the top surfaces **103** of the pillars **101**. The pillars **101** may then be aligned with the fluid channels **112** of the dispenser **110** and liquids containing different potential candidate drugs can pass through the different vertical fluid channels **112** and to the sample surfaces of the pillars **101**. Potential interactions or reactions between the different candidate drugs and the proteins can take place within these reaction chambers formed by the pillars **101** and the fluid chambers **112**. A predetermined amount of time may be permitted to elapse to allow any reactions or interactions to occur. In some embodiments, the time may be 1 minute or more. In other embodiments, the elapsed time may surpass 30 minutes or more. After any reactions or interactions are permitted to occur, the chip **105** and the dispenser **110** may be separated from each other. Discrete liquid samples may be present on the top surfaces **103** of the chip **105** after the chip **105** is separated from the dispenser **110**. Then, the sample surfaces **103** of the pillars **101** may be washed. The sample surfaces **103** may then be analyzed to determine which, if any, of the potential

candidate drugs bind to the proteins on the top surfaces **103** of the pillars **101**. To help identify the candidate drugs, the candidate drugs may have different fluorescent tags bound to them prior to being on the sample surfaces **103**.

In another embodiment, the fluid channels **112** may have liquids with capture agents that are to be bound to the top surfaces of the pillars **101**. The pillars **101** may be introduced in the fluid channels **112**, thereby forming a small reaction chamber together with the inner fluid channel walls, the molecules in the liquid are thereby given the opportunity to react or bind (e.g., without leaving a distinct deposit of liquid on the pillar). Alternatively, the liquids can be deposited on the pillars **101** and the capture agents may bind to the top surfaces **103** of the pillars **101**. The dispenser **110** and the chip **105** can be separated and the capture agents bound to the top surfaces may be used to capture analytes for analysis.

The assemblies may include one or more passive valves. A passive valve stops the flow of liquid inside or at the end of a capillary using a capillary pressure barrier that develops when the characteristics of the capillary or mini channel changes, such as when the capillary or channel cross-section changes abruptly, or when the materials of structures defining the fluid channels change abruptly. Passive valves are discussed in P. F. Man et al., "Microfabricated Capillary-Driven Stop Valve and Sample Injector," IEEE 11th Annual Int. MEMS Workshop, Santa Clara, California, Sept. 1999, pp. 45-50, and M. R. McNeely et al., "Hydrophobic Microfluidics," SPIE Conf. on Microfluidic Devices and Systems II, Santa Clara, California, Sept. 1999, vol. 3877, pp. 210-220. These publications are herein incorporated by reference for all purposes. Passive valves are unlike active valves which completely close off a fluid channel with a physical obstruction.

In an illustrative example of how an assembly with a passive valve can be used, the structures of a chip can be inserted into respective fluid channels in a dispenser. Each fluid channel can have one, two, or three or more passive valves. For instance, each fluid channel may have a passive valve that is formed by an abrupt structural change in the geometry of a fluid channel. For example, the walls of a fluid channel may form a step structure. When a liquid encounters the step structure at a predetermined pressure, the liquid stops flowing.

Passive valves can also be formed when the structures containing the sample surfaces are within or are positioned at the ends of the fluid channels. For example, a pillar may be inserted into a fluid channel so that there is a space between the side surfaces of the pillar that is in the fluid channel and the fluid channel walls around the pillar. The portion of

the fluid channel where the pillar resides may have an annular configuration. As liquid flows towards the pillar, the geometry of the fluid channel changes from a cylindrical configuration to an annular configuration. At a predetermined pressure, the liquid stops flowing at this geometry change. Additional pressure is needed to cause the liquid to flow past this geometry change. Different pressures may be applied to initiate the flow of liquid past each of the passive valves in the fluid channel. For example, two different levels of pressure may be applied to a fluid in a fluid channel to move a liquid past two different passive valves.

In one specific example of an assembly with a dispenser using one or more passive valves, a chip including pillars is used with a dispenser containing a plurality of fluid channels. The pillars may be inserted into the fluid channels and the chip may be brought into contact with the dispenser. Before or after insertion, a first pressure is applied to the liquids in the fluid channels to push the fluid samples to, but not substantially past, the first passive valve. A second pressure is then applied to the fluid samples to push the samples past the first passive valve so that the liquids are in contact with the pillars. The samples do not pass the second passive valve, which is defined by the pillar and the channel walls. After the liquids in the fluid channels contact the sample surfaces, the pressure applied to the liquids is decreased. Then, the dispenser and the chip are separated from each other to separate the sample surfaces from the bulk of the liquids in the fluid channels. In this step, the pillars are withdrawn from the fluid channels and liquid samples may remain on the sample surfaces. Withdrawing the pillars from the fluid channels may stop any events that may be occurring at the sample surfaces. Alternatively, reactions can still occur after the pillars are withdrawn from the fluid channels and reactions can stop after a washing step is performed. After liquid samples are transferred to the sample surfaces, processes such as evaporation and the formation of an air-liquid interface will have little or no adverse effect on the deposited components in the liquid samples. Any residual solvent or material on the sample surface may be rinsed away leaving the desired components on the sample surfaces.

In other embodiments, the structures may be inserted into the fluid channels until contact is made with liquids within respective channels. In these embodiments, added pressure need not be applied to the fluids in the fluid channels to bring the fluids in contact with the sample surfaces of the structures.

The dispensers according to embodiments of the invention have a number of advantages. For instance, unlike conventional ring-pin dispensers, embodiments of the invention can deliver a large number of liquids to the sample surfaces in parallel. For

example, in embodiments of the invention, 10,000 or more fluid channels can be used to dispense 10,000 liquid samples. In comparison, conventional ring-pin dispensers may have only about 30 ring pins per assembly. Also, unlike a capillary pin dispenser that can potentially physically touch a sample surface thus potentially damaging the dispenser and the sample surface, many of the described dispenser embodiments do not come in contact with the sample surface. Moreover, unlike many conventional dispensers, the assembly embodiments of the invention can reduce the likelihood of forming an air-liquid interface, since droplets are not formed when liquid is transferred from a dispenser to a chip. As the volume of a drop gets smaller, the surface to volume ratio of the drop gets larger leading to problematic interactions between the molecules in the liquid that are to be transferred to the sample surface and the air-liquid interface of the drop. In embodiments of the invention, droplets of liquid need not be formed, thus minimizing the formation of a liquid sample with a gas/liquid interface with a high surface to volume ratio.

Specific examples of assemblies using passive valves may be described with reference to FIGS. 10-14. Referring to FIGS. 10 and 11, a liquid **270** is placed in the fluid channel **112** in a dispenser **118**. A first dispenser portion **120(a)** may comprise a hydrophilic material and a second dispenser portion **120(b)** may comprise a hydrophobic material. The fluid channel **112** is then aligned with a pillar **101** on a base **105a** of a chip **100** and the pillar **101** is inserted into the fluid channel **112**. As shown in FIG. 11, the dispenser **110** and the chip **100** are in contact with each other when the pillar **101** is inserted into the fluid channel **112**. Before or after the pillar **101** is inserted into the fluid channel **112**, a first pressure is applied to the liquid **270**. The first pressure may be greater than atmospheric pressure. The liquid **270** flows to, but not past, a first passive valve **114** defined within the fluid channel **112**. The passive valve **114** may be formed by an abrupt change in the cross-sectional area of the fluid channel **112**. Alternatively or additionally, the passive valve **114** may be formed by an abrupt change in the material of the fluid channel walls (e.g., hydrophilic to hydrophobic). Regardless of the particular form that the passive valve **114** takes, the passive valve **114** prevents the liquid **270** from flowing out of the fluid channel **112** at the pressure P1.

Referring to FIG. 12, after the pillar **101** is inserted into the fluid channel **112**, a pressure P2 may be applied to the liquid **270**. The pressure P2 may be greater than the pressure P1. The applied pressure P2 causes the liquid **270** to flow past the first passive valve **114** and onto a material at the top surface **103** of the pillar **101** and to a second passive valve

115 defined by the top surface 103 of the pillar 101 and the surrounding walls of the fluid channel 112.

Referring to FIG. 13, the abrupt change in geometry occurs at a fluid channel region 109 near the top surface 103 of the pillar 101. In this example, this region 109 of the fluid channel 112 has an annular shape due to the presence of the pillar 101. The liquid 270 reacts with the material on the top surface 103 of the pillar 101. Alternatively, the liquid 270 and components in the liquid 270 may simply deposit on the top surface 103 of the pillar 101.

After the liquid 270 is on the top surface 103 of the pillar 101, the majority of the liquid 270 may be separated from the pillar 101. For example, referring to FIG. 14, a pressure less than the pressure P2 (e.g., less than atmospheric pressure) is applied to the liquid 270 so that the bulk of the liquid 270 flows upward while leaving a portion of the liquid 270 on the pillar 101. In other embodiments, the chip 105 and the dispenser 110 may be separated from each other to separate the bulk of the liquid 270 from the liquid deposited on the pillar 101. The pillar 101 may be withdrawn from the fluid channel 112 and the bulk of the liquid 270 may be retained in the fluid channel 112 of the separated dispenser 110. In some embodiments, separation of the pillar 101 from the fluid channel 112 may stop any interaction between the liquid and any material at the top surfaces of the pillar 101. In these embodiments, a pressure less than pressure P2 is not needed to separate the bulk of the fluid 270 from the pillar 101. After the dispenser 110 is separated from the chip, the top surface of the pillar 101 may be rinsed or flushed with another liquid. The rinsing or flushing step can stop any interactions between the liquid and any material at the top surfaces of the pillar 101, if the prior separation of the chip 105 and the dispenser 110 does not stop the interactions taking place.

FIG. 15 illustrates an assembly embodiment with a dispenser with a passive valve. The dispenser 110 has a fluid channel 112 having a first channel section 112a communicating with a second channel section 112b. The first channel section 112a is wider than channel section 112b. In this example, both the first channel section 112a and the second channel section 112b terminate in a shoulder 113 which forms a restriction between the first channel section 112a and the second channel section 112b. The restriction (or a preventative means for preventing the flow of liquid 270) functions as a passive valve 114. The internal walls of the channel 112 may have a hydrophobic surface 230. The top surface 103 of the pillar 101 may be a hydrophilic surface 234.

In the embodiment shown in FIG. 15, the liquid **270** may be deposited on the pillar **101** in the same or different manner as the processes described with reference to FIGS. 10-14. For instance, the pillar **101** may be inserted into or positioned at the end (e.g., exactly at the end of the fluid channel or just outside of the end of the fluid channel) of the fluid channel **112** of the dispenser **110**. The dispenser **110** may or may not contact the chip **105** during the process of depositing liquid onto the pillar **101**. When the flow of liquid **270** is stopped at the first passive valve, the liquid **270** may be at a pressure P_1. A second pressure P_2, which is greater than the first pressure P_1, is subsequently applied to the liquid **270** to force the liquid **270** through and past the first passive valve **114** until it contacts the hydrophilic surface **234** on the pillar **101** that lies within the fluid channel **112**. The upper portion of the pillar **101** and the surrounding fluid channel **112** may form a second restriction that forms a second passive valve. Alternatively, the hydrophilic surface **234** on top surface **103** of pillar **101** in combination with the hydrophobic surfaces **230** on the walls of second channel section **112b** and on side **104** of pillar **101** functions as the second passive valve. In both instances, the flow of the fluid **270** stops at the upper surface of the pillar **101**. The top surface of the chip base **105a** may also be a hydrophobic surface **230**. The bottom surface of the dispenser **110** may also be a hydrophobic surface **230**.

The hydrophilic surface **234** may be produced according to any suitable process and may include any suitable materials. For example, silicon oxide (e.g., SiO_2), and polymers terminating in hydrophilic groups (e.g., OH or COOH) may be used to form a hydrophilic surface **234**. The hydrophilic surface **234** on top of the pillars **101** may be produced according to procedures disclosed in U.S. Patent Application No. 09/115,397, which is assigned to the same assignee as the present invention and is herein incorporated by reference in its entirety for all purposes.

FIG. 16 shows another assembly embodiment. This embodiment is similar to the embodiment shown in FIG. 15. However, in this example, the second channel section **112b** is on top of the first channel section **112a** and the liquid **270** passes through the second channel section **112b** before entering the first channel section **112a**. The walls of the channel **112** in this example have the hydrophilic surface **234**. A first pressure P_1 is applied to the liquid **270** to force the liquid **270** through the second channel section **112b** up to, but not past, a first passive valve **240**. In FIG. 16, the abrupt enlargement defines the first passive valve **240**. The abrupt enlargement is an instantaneous increase of the width of the fluid channel **112** defines a shoulder **113**. A second pressure P_2, which may be greater than the first

pressure P_1, is subsequently applied to the liquid **270** to push the liquid **270** through and past the first passive valve **240** until contacting the hydrophilic surface **234** of the pillar **101**. The liquid **270** encounters a restriction defined by the pillar **101** when the pillar **101** is in the channel **112**. This restriction may function as a second passive valve. Alternatively or additionally, the hydrophilic surface **234** on top surface **103** and on internal wall of first channel section **112a** in combination with the hydrophobic surface **230** on the pillar chip **105**, including on the sides **104** of the pillar **101** may function as the second passive valve. The restriction prevents the flow of liquid **270** out of fluid channel **112** and onto the pillar chip **105**.

FIGS. 17(a) to 17(d) show cross sections of assembly embodiments including a chip with a pillar having a concave side surface. A sequence of steps that may be used to deposit a liquid sample onto a sample surface of a pillar may be described with reference to FIGS. 17(a) to 17(d).

FIG. 17(a) shows a pillar **322** on a base **320** of a chip. The pillar **322** includes a sample surface **322(a)** and a side including a concave portion **322(b)** between an upper non-concave portion and a lower non-concave portion. A first edge **322(c)** and a second edge **322(d)** define the upper non-concave portion. A dispenser **301** is above the chip, and a fluid channel **341** in the dispenser **301** is aligned with and is over the pillar **322**. A liquid **340** is in the fluid channel **341** and a step structure **303** prevents the liquid **340** from passing to the pillar **322**. The step structure **303** may function as a first passive valve that stops the flow of liquid at a pressure P1.

FIG. 17(b) shows the liquid **340** contacting the sample surface **322(a)** of the pillar **322**. In this example, a pressure P2 is applied to the liquid **340** so that the liquid sample flows past the step structure **303** of the dispenser **301**. The pressure P2 in this example is greater than the pressure P1. At the pressure P2, the liquid **340** may flow until it encounters the edge surfaces **322(c)**, **322(d)** of the upper non-concave portion. As shown in FIG. 17(b), the flow of the liquid **340** may stop at the upper edge **322(c)** of the pillar. The edge **322(c)** and a portion of the wall defining the fluid channel **341** may form a second passive valve that stops the liquid **340** from flowing past the edge **322(c)** at the pressure P2.

Alternatively or additionally, as shown in FIG. 17(c), the flow of the liquid **340** may stop at the bottom edge **322(d)** of the upper non-concave portion of the pillar **322** when the pressure P3 is applied to the liquid **340**. The edge **322(d)** and the surrounding wall may form a third passive valve that stops the liquid **340** from flowing past the edge **322(d)**.

The pressure P3 may be greater than the pressures P1 and P2. Although pressure is applied to the liquid **340** in the examples shown in FIGS. 17(b) and 17(c), in other embodiments, a higher pressure need not be applied to the liquid **340** to bring the liquid **340** in contact with the sample surface **322(a)** of the pillar **322**. For instance, the pillar **322** and/or the dispenser **301** may move toward the other until they contact each other. Accordingly, in some embodiments, the sample surface and a liquid in a fluid channel can contact each other without applying additional pressure to the liquid **340**.

Advantageously, the pillar **322** shown in FIGS. 17(b) and 17(c) can, when in a fluid channel, form two passive valves proximate the upper portion of the pillar **322**. Having two passive valves instead of one to stop the flow of liquid at the top portion of the pillar **322** helps to ensure that a substantial amount of the liquid **340** does not flow down the sides of the pillar **322**. The flow of liquid **340** down the sides of the pillar **322** is further minimized and the likelihood that the liquid sample will flow to an adjacent sample surface is also minimized. This further reduces the likelihood of cross-contamination between samples on different sample surfaces.

Referring to FIG. 17(d), after the liquid **340** contacts the sample surface **322(a)** of the pillar **322**, a portion **327** of the liquid **340** may deposit on the sample surface **322(a)**, while the bulk of the liquid **340** may be separated from the sample surface. This may be accomplished by applying a lower pressure to the liquid **340**. For example, a pressure P4, which may be less than the pressures P2 and P3, may be applied to the liquid **340**. The lower pressure causes the liquid **340** to flow upward into the fluid channel **341**. Alternatively or additionally, the dispenser **301** and the chip may be separated from each other by moving the chip and/or the dispenser away from the other. If a portion **326** of the liquid sample does not deposit on the sample surface, it can flow down a side of the pillar **322** without flowing to a liquid sample **327** on an adjacent pillar **333**. Cross-contamination between samples on adjacent surfaces is thus minimized.

The dispensers used in embodiments of the invention may be in any suitable form. For example, FIGS. 18 to 23 illustrate cross-sections of portions of various types of dispensers. FIG. 18 illustrates a nozzle that dispenses droplets similar to an inkjet (micro-drop dispenser). FIG. 19 illustrates a metal pin for dispensing liquid onto the pillars. FIGS. 21 and 23 show dispensers with necks **801**. The necks **801** can correspond to the ends of the fluid channels and can be used to pierce through an outer surface of a drop that is on a sample surface. A liquid sample can be delivered through a neck in the dispenser and into the

interior of a drop of liquid. This minimizes contact between the liquid in the dispenser and the air. The necks **801** can also be used as a barrier to minimize cross-contamination between liquids in adjacent fluid channels in the dispenser.

As shown in FIGS. 18, 19, and 23, in some embodiments, portions of the fluid channel of a dispenser that are proximate to a pillar **306** may be smaller than the cross-sectional area of the pillar **306** so that the pillar **306** may not fit within the fluid channel of the dispenser. However, as shown in FIG. 22 and in many of the previous Figures, portions of a fluid channel of a dispenser may have a larger cross-sectional area than a pillar so that the pillar is removably insertable into the fluid channel.

FIGS. 24 to 25 illustrate a dispenser **130** for use with a chip **131** that includes elongated pillars **132**. Dispenser **130** includes elongated (e.g., in an x- or y- direction) dispenser nozzles **133** that engage or cooperate with the elongated pillars **132**. The elongated dispenser nozzles **133** each have a neck that can be used to prevent cross-contamination between adjacent nozzles **133**. Channels **134** are defined on the chip **131** by channel defining walls **135**. The walls **135** of the chip **131** can contact and support the dispenser **130** while it is on the chip **131**.

FIGS. 26 to 27 illustrate an assembly with a specific type of dispenser. The dispenser may be referred to as a fluid addressing adaptor device **140**. The adaptor device **140** may include fluid storage wells **142** that communicate with fluid channels **143** (defined by walls **160**) via flow paths **144**. The fluid flow paths **144** extend horizontally in the adaptor device **140**. The pillars **164** on the chip **158** may be aligned with, inserted into, holes **145** in the bottom walls defining the fluid channels **143**. The upper regions of the pillars **164** may protrude through the pillar holes **145**. Unlike many of the previously described embodiments, liquids flowing through the fluid channels **143** may flow horizontally and may contact the top surfaces of the pillars **164** since the top surfaces are exposed to the flowing fluids. Different fluids may flow from respectively different storage wells **142**. These different fluids may flow through the different fluid channels **143** and may contact the top sample surfaces of the pillars. After contacting the sample surfaces of the pillars **164**, the liquid flowing downstream of the pillars **164** may pass to a fluid outlet **141**. Embodiments of the invention can be used with microfluidic devices such as the "lab on a chip" type devices.

Furthermore, the dispenser or another device may be used in conjunction with other external devices such as a mass spectrometer. External devices such as these may be used to analyze reactions or interactions at the sample surfaces. Such external devices may

be downstream of the sample surfaces. Further details regarding the use of assemblies with such external devices are in U.S. Patent Application No. _____, entitled "Microfluidic Devices and Methods", by Paul Jedrzejewski et al. filed concurrently herewith (Attorney-Docket No. 020144-001510). This application is herein incorporated by reference in its entirety for all purposes and is assigned to the same assignee as the present invention.

FIG. 28 illustrates another type of dispenser that may be referred to as an "anti-interference adaptor" **172**. The anti-interference adaptor **172** may include a plurality of holes **180** at the bottom surface of the adaptor **172**. In some embodiments, the anti-interference adaptor **172** may be translucent or transparent. As shown in FIG. 29, the pillars **178** of a chip **170** may be inserted into the holes **180** and may be adjacent to the chip **170**. The adaptor **172** may include a fluid channel in the form of a flow chamber and a fluid inlet **174** and a fluid outlet **176**. The flow chamber contains a liquid that contacts top sample surfaces of the pillars **178** of the chip **170**. The liquid in the flow chamber flows horizontally and contacts a plurality of the sample surfaces substantially simultaneously. By using the flow-cell adaptor **172**, a fluid with or without analytes can be quickly introduced to the multiple sample surfaces. Non-specific binding of analytes to the sides of the pillars **178** is minimized as the fluid primarily contacts the top regions of the pillars **178**. After the sample surfaces of the pillars **178** contact the fluids, the anti-interference adaptor **170** can remain adjacent to the chip **170** while the characteristics of the samples on the sample surfaces are detected.

External devices (not shown) such as optical devices may be used to detect chemical reactions between the material flowing through the chamber and any materials on the top surfaces of the pillars **178**. For example, a light signal **180** can be directed to the samples on the surfaces of the pillars **178** and the reflected signal can be detected to determine if a reaction has occurred at the sample surface.

FIG. 31(a) shows another assembly embodiment. Referring to FIG. 31(a), this embodiment includes a chip **191** having a base **192** with troughs **198** separated by a support **196**. A number of pillars **190** are on the bottom surfaces of the troughs **198**. Each of the pillars **190** may have a height that is substantially equal to the depth D of the trough **198** that it is in. In other embodiments, the pillars may have a height that is less than the depth D of the trough that they are in. A cover **194** is on the base **192** of the chip **191** and the troughs **198** can contain fluids such as liquids or gases that contact the sample surfaces on the pillars **190**. In this example, there are two troughs, and each trough may contain different fluids. In

other embodiments, there may be more or less than two troughs. For example, in some embodiments, there may be six troughs (or more) with 250 pillars (or more) in each trough.

FIG. 31(b) shows a top view of the assembly embodiment shown in FIG. 31(a) with the sidewalls defining the troughs **198** being shown by invisible lines. Fluids may be introduced through fluid inlets **197(a)** in the cover **194** at first ends of the troughs **198**. The cover **194** may be considered a dispenser, since fluids are being dispensed onto the sample surfaces on the pillars **190**. The fluids then pass through the troughs **198** to the opposite ends and pass out of fluid outlets **197(b)** in the cover **194**. In other embodiments, the fluid inlets and/or the fluid outlets may be provided in the base **192**. As the fluids pass through the troughs **198**, the fluids contact the top sample surfaces of the pillars **190** and any substances at those top sample surfaces. After the fluids contact the top sample surfaces of the pillars **190**, the top sample surfaces may be analyzed to determine if any interactions or reactions have taken place. The analysis may take place with or without the cover **194** on the base **192**.

The embodiment shown in FIGS. 31(a) and 31(b) has a number of advantages. For example, unlike some of the previously described embodiments, the pillars **190** on the chip **191** need not be aligned with holes in a dispenser. Fluids can be introduced to the top sample surfaces of the pillars **190** without a precise aligning step. Liquids or gases containing different components may contact a plurality of sample surfaces substantially simultaneously. Accordingly, procedures such as assays can be performed quickly using embodiments such as those shown in FIGS. 31(a) and 31(b).

Any of the described dispenser/sample chip combinations can be used together in a single process. For example, in one exemplary embodiment, dispensers that have passive valves (e.g., as shown in FIGS. 10-17) can be used to deposit different capture agents on the top surfaces of the pillars of the sample chips. After the capture agents are bound to the top surfaces of the pillars, dispensers such as the ones shown in FIGS. 26-30 may be used to dispense analyte containing liquids so that they contact the capture agents bound to the top surfaces of the pillars.

The terms and expressions which have been employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding equivalents of the features shown and described, or portions thereof, it being recognized that various modifications are possible within the scope of the invention claimed. Moreover, any one or more features of any embodiment of the invention may be combined with any one or more other features of any other embodiment of the

invention, without departing from the scope of the invention. For example, any specifically described sample structure embodiments in FIGS. 2-6 may be used with the assemblies shown in FIGS. 8-31 without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A chip comprising:

a) a base including a non-sample surface; and

b) at least one structure, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive a sample from a dispenser.

2. The chip of claim 1 comprising a plurality of the structures.

3. The chip of claim 2 wherein the plurality of the structures are arranged as an array.

4. The chip of claim 1 wherein the chip comprises at least one of silicon, silicon oxide, polymeric materials, or glass.

5. The chip of claim 1 wherein each structure further comprises a metal, a metal oxide, a polymeric material, or gold on the pillar.

6. The chip of claim 1 wherein each structure comprises an affinity structure on the pillar.

7. The chip of claim 1 wherein each structure comprises a monolayer on the pillar.

8. The chip of claim 1 further comprising the sample, wherein the sample is a liquid sample.

9. The chip of claim 1 further comprising the sample, wherein the sample is a liquid sample and wherein the liquid sample interacts with the sample surface.

10. The chip of claim 1 wherein sides of each structure are hydrophobic.

11. The chip of claim 1 wherein sides of each structure are hydrophilic.

12. The chip of claim 1 wherein the pillar includes a concave portion.

13. The chip of claim 1 wherein the pillar has an aspect ratio greater than about 0.25.

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14. The chip of claim 1 wherein the pillar has a fluid passage extending in an axial direction through the pillar.

15. The chip of claim 1 wherein the pillar has a width less than about 1.0 mm.

16. The chip of claim 1 wherein the base further comprises a trough defined by walls and a bottom, wherein each structure extends from the bottom of the trough.

17. The chip of claim 1 wherein the base further comprises a trough defined by walls and a bottom, wherein each structure extends from the bottom of the trough and has a height less than or equal to a depth of the trough.

18. The chip of claim 1 wherein the non-sample surface has different properties than the sample surface.

19. The chip of claim 1 wherein the non-sample surface has the same properties as the sample surface.

20. An assembly adapted to process fluids, the assembly comprising:

a) a dispenser comprising a body and at least one fluid channel defined in the body, each fluid channel being adapted to dispense a fluid on one or more of the sample surfaces; and

b) a chip comprising (i) a base including a non-sample surface, and (ii) at least one structure, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive the fluid from the dispenser.

21. The assembly of claim 20, wherein each fluid channel is cooperatively structured to receive one or more of the structures of the chip.

22. The assembly of claim 20 wherein each fluid channel includes a passive valve.

23. The assembly of claim 20 wherein each fluid channel has a passive valve formed by a first channel portion and a second channel portion, the first channel portion being wider than the second channel portion.

24. The assembly of claim 23 wherein the first channel portion is above the second channel portion.

25. The assembly of claim 20 wherein the dispenser is an ink-jet type dispenser.

26. The assembly of claim 20 wherein at least one fluid channel is horizontal, and wherein the structures of the chip are insertable through holes in the bottom walls defining the at least one fluid channel.

27. The assembly of claim 20 wherein the base further comprises a trough defined by walls and a bottom, and each structure extends from the bottom of the trough and has a height less than or equal to the depth of the trough.

28. A method of processing fluids, the method comprising:

a) supplying a fluid in a fluid channel in a dispenser; and

b) dispensing the fluid on one or more structures on a base of a chip, wherein each structure comprises a pillar and includes a sample surface that is elevated with respect to the non-sample surface.

29. The method of claim 28 wherein the dispenser comprises a plurality of fluid channels and wherein supplying the fluid comprises:

supplying a plurality of liquids to respective fluid channels in the plurality of fluid channels in the dispenser.

30. The method of claim 28 wherein the dispenser comprises a plurality of fluid channels and supplying the fluid comprises:

supplying a plurality of different liquids containing different components to respective fluid channels in the plurality of fluid channels in the dispenser.

31. The method of claim 30 wherein the different components are respectively different analytes or different capture agents.

32. The method of claim 28 wherein the fluid comprises a reagent.

33. The method of claim 28 wherein the dispenser comprises a plurality of fluid channels and supplying the fluid comprises supplying a plurality of liquids containing different components to respective fluid channels in the plurality of fluid channels, and wherein the method further comprises:

binding the different components to the sample surfaces.

34. The method of claim 28 wherein the dispenser comprises a plurality of fluid channels and supplying the fluid comprises supplying a plurality of liquids to respective fluid channels in the plurality of fluid channels, and wherein the method further comprises, after dispensing:

depositing a plurality of liquid samples on the sample surfaces of the chip.

35. The method of claim 28 wherein the dispenser comprises a plurality of fluid channels and supplying the fluid comprises supplying a plurality of liquids to respective fluid channels in the plurality of fluid channels, and wherein dispensing comprises:

applying a first pressure to the plurality of liquids in the fluid channels in the dispenser to push the fluid to first passive valves in the fluid channels;

placing the one or more sample surfaces of the chip within the fluid channels or at ends of the fluid channels, and engaging the dispenser with the chip;

applying a second pressure to the plurality of liquids in the fluid channels to push the liquids past the first passive valves and into contact with the samples surfaces, the second pressure being greater than the first pressure; and

applying a third pressure to the plurality of liquids in the fluid channels, the third pressure being less than the second pressure.

36. The method of claim 28 wherein dispensing takes place without forming droplets of liquid.

37. A method of processing fluids, the method comprising:

a) supplying a plurality of liquids to respective fluid channels in a dispenser, wherein each of the fluid channels includes a passive valve and wherein the flow of each liquid in each fluid channel stops at the passive valve;

b) aligning sample surfaces of a plurality of structures with the plurality of fluid channels, wherein each structure comprises a pillar; and

c) contacting the sample surfaces and the liquids in the fluid channels while the sample surfaces are in, or are positioned at ends of, the fluid channels.

38. The method of claim 37 wherein the method further comprises:

applying pressure to the liquids in the fluid channels to force the liquids past the passive valve.

39. The method of claim 37 wherein the liquids within the different fluid channels contain respectively different capture agents.

40. The method of claim 37 wherein each of the pillars has an aspect ratio greater than about 0.25.

41. The method of claim 37 wherein the structures are present in a chip, and wherein the method further comprises, after d):

allowing the sample surfaces to remain in or at the ends of the fluid channels for a predetermined amount of time; and then

separating the chip and the dispenser.

42. The method of claim 37 wherein the structures are present in a chip, and wherein liquids in the fluid channels contain respectively different components.

43. The method of claim 37 wherein the method further comprises, prior to a):

binding a plurality of capture agents to the sample surfaces.

44. The method of claim 37 further comprising analyzing the samples on the sample surfaces after d).

45. The method of claim 37 further comprising: after d)

processing substances on the sample surface using the deposited liquid samples;

separating the sample surfaces and the dispenser;

placing a cover chip having a plurality of fluid passages positioned over and aligned with the sample surfaces; and

transferring the processed substances to an analysis device through the fluid passages in the cover chip.

46. A chip comprising:

a) a base including a non-sample surface; and

b) a plurality of structures in an array on the base, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive a sample from a dispenser to be processed or analyzed while the sample is on the sample surface.

47. The chip of claim 46 wherein the chip further comprises a plurality of proteins bound to the sample surfaces.

48. The chip of claim 46 wherein the chip further includes a plurality of liquid samples on the sample surfaces.

49. The chip of claim 46 wherein each structure includes an affinity structure on the pillar.

50. The chip of claim 46 wherein the pillars and the base comprise silicon.

51. The chip of claim 46 wherein the sides of each structure are hydrophobic.

52. An assembly comprising:

a) a chip comprising: i) a base including a non-sample surface; and ii) a plurality of structures in an array on the base, each structure comprising a pillar and a sample surface that is elevated with respect to the non-sample surface and is adapted to receive a sample to be processed or analyzed while the sample is on the sample surface; and

b) a dispenser including a plurality of fluid channels, each fluid channel including a passive valve.

53. The assembly of claim 52 wherein each fluid channel includes two or more passive valves when the structures of the chip are aligned with the fluid channels of the dispenser.

54. The assembly of claim 52 wherein the passive valve is defined by an instantaneous change in the geometry of the fluid channel.

55. The assembly of claim 52 wherein at least a portion of the walls defining the fluid channels are hydrophobic.

56. The assembly of claim 52 wherein at least a portion of the walls defining the fluid channels are hydrophilic.

57. The assembly of claim 52 wherein each pillar has an aspect ratio greater than about 0.25.

58. The assembly of claim 52 wherein each structure includes an affinity structure on the pillar.

59. The assembly of claim 52 wherein each structure includes a metal layer or an oxide layer on the pillar.

60. The assembly of claim 52 wherein the pillar has an aspect ratio greater than about 1.

61. The assembly of claim 52 wherein the liquid samples comprise proteins.

62. The assembly of claim 52 wherein the pillar comprises silicon.

63. The assembly of claim 52 wherein the dispenser includes a translucent or transparent material.

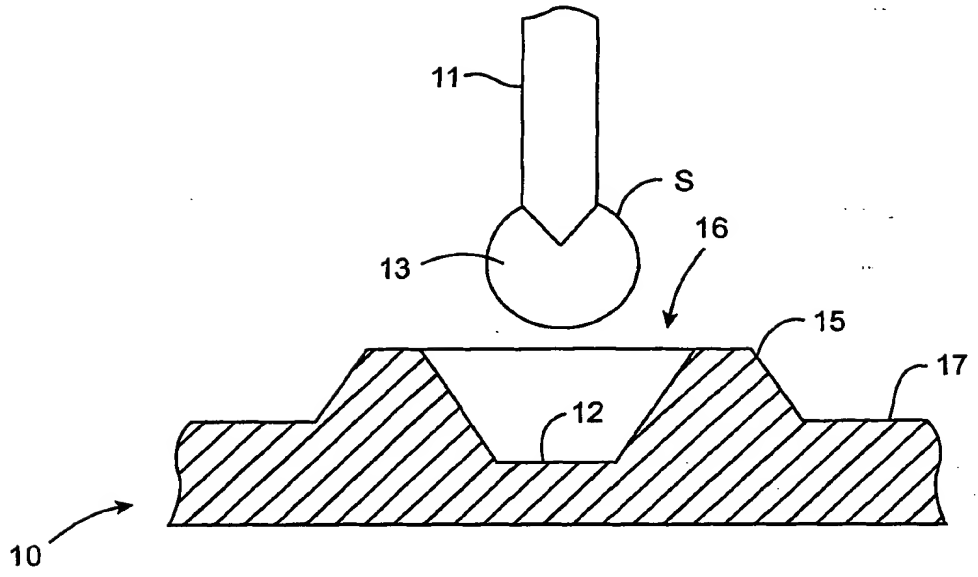


FIG. 1(a)

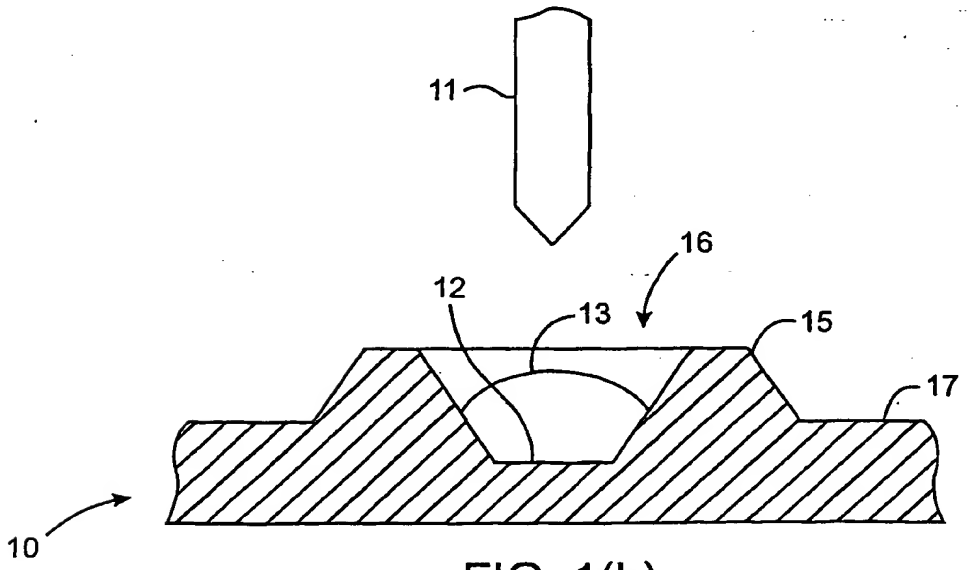


FIG. 1(b)

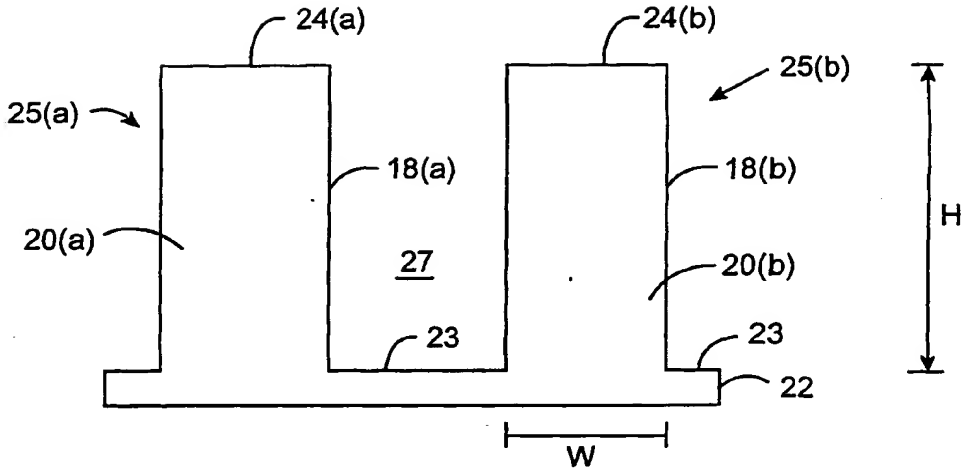
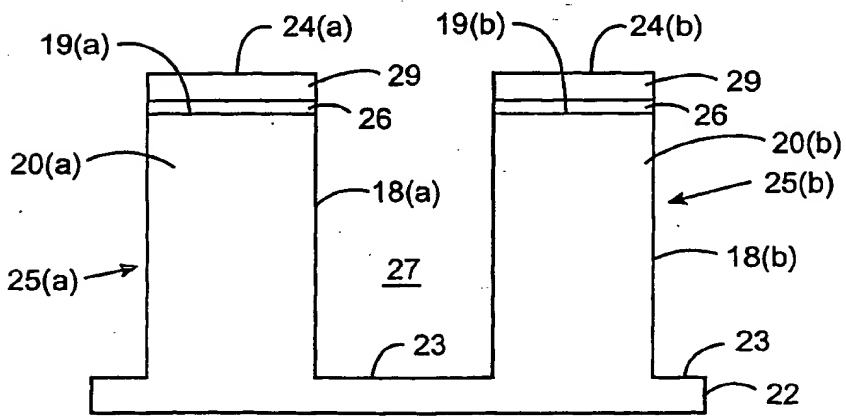

24(a)
24(b)
25(b)
25(a)
18(a)
18(b)
H
20(a)
27
20(b)
23
23
22
W

FIG. 2(a)


19(a)
24(a)
19(b)
24(b)
29
29
26
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18(b)
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25(a)
23
23
22

FIG. 2(b)

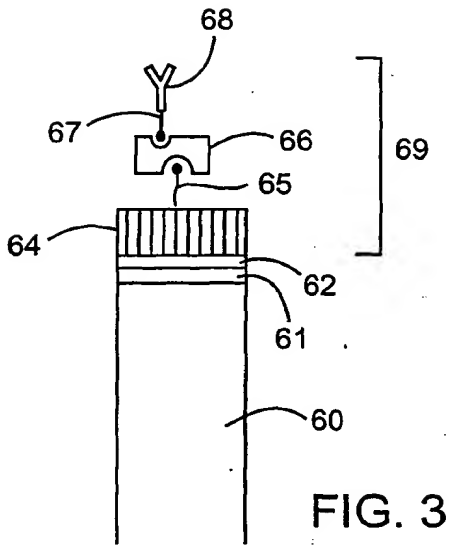


FIG. 3

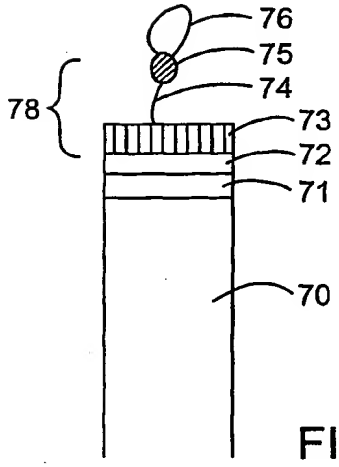


FIG. 4

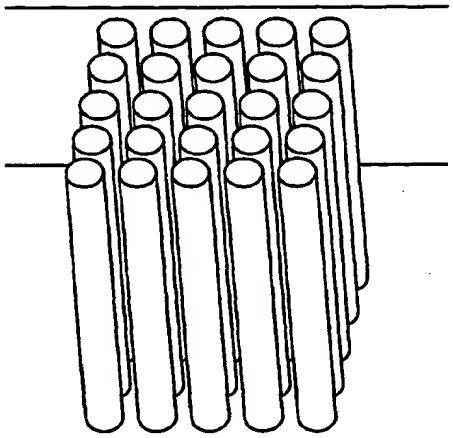


FIG. 5

FIG. 6(a)

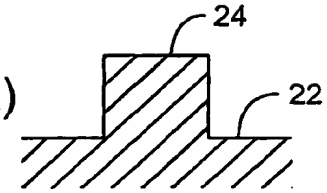


FIG. 6(b)

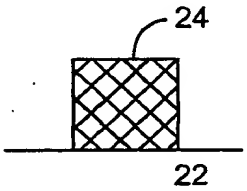
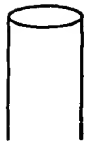


FIG. 6(c)



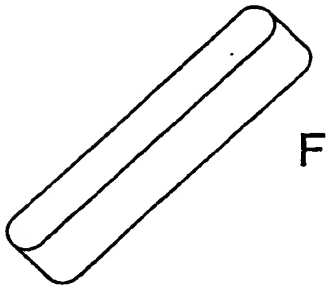
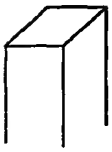


FIG. 6(d)

FIG. 6(e)



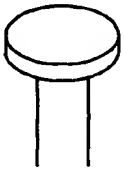
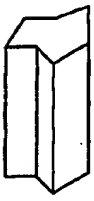


FIG. 6(f)

FIG. 6(g)



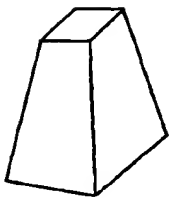


FIG. 6(h)

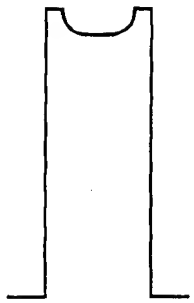


FIG. 6(i)

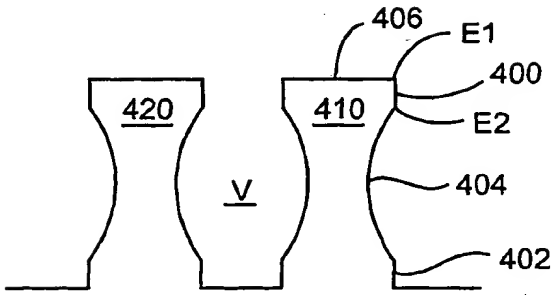


FIG. 6(j)

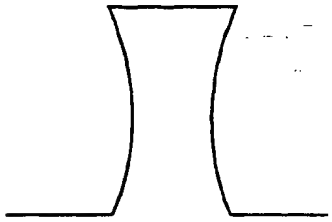


FIG. 6(k)

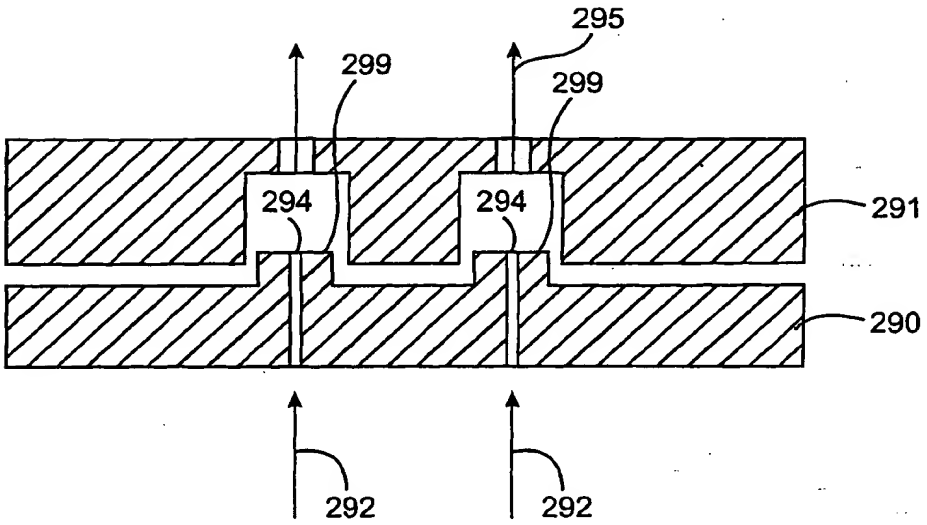


FIG. 6(I)

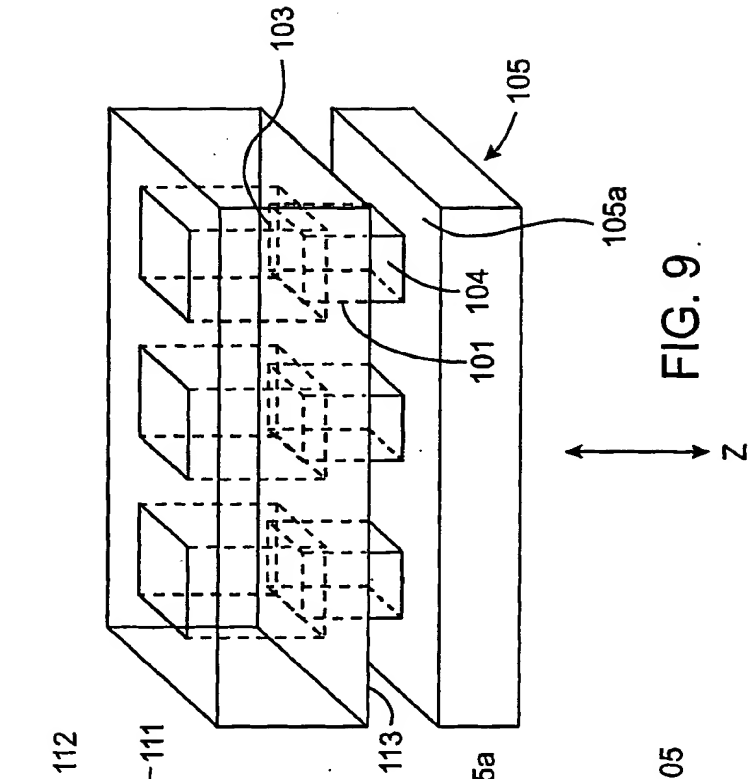


FIG. 9

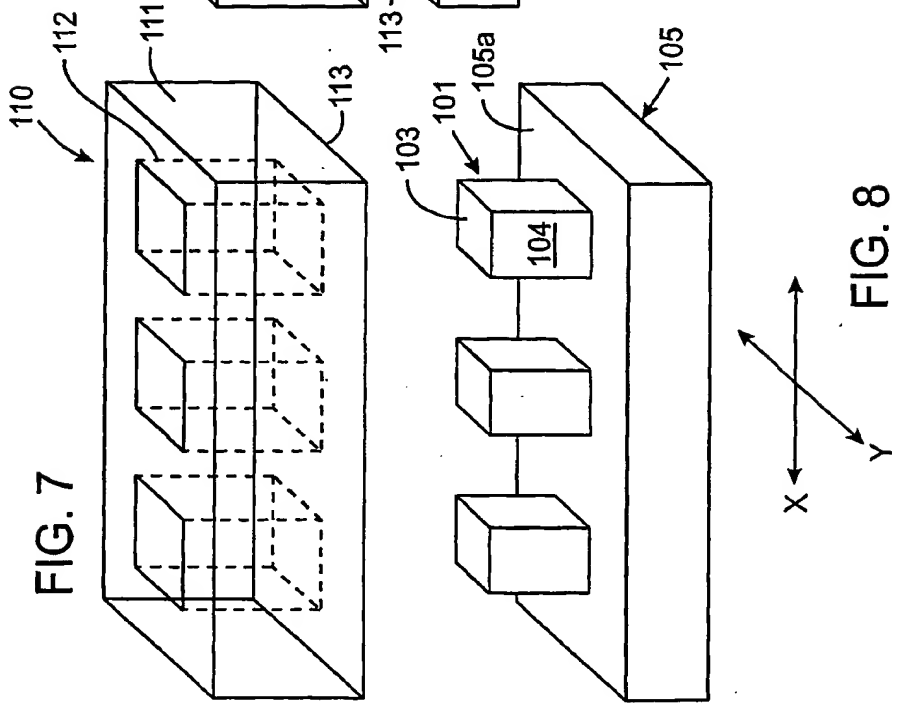


FIG. 8

FIG. 7

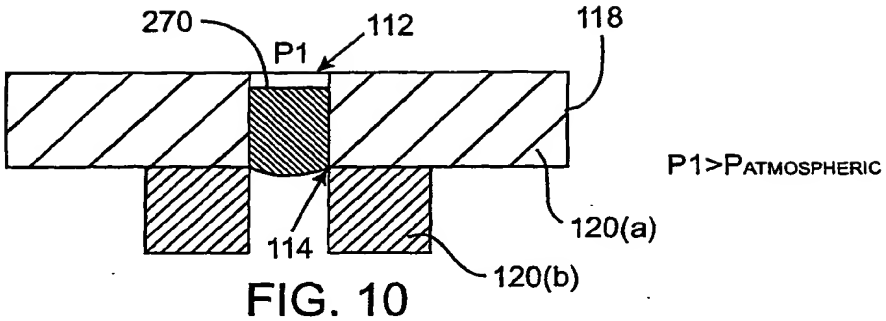


FIG. 10

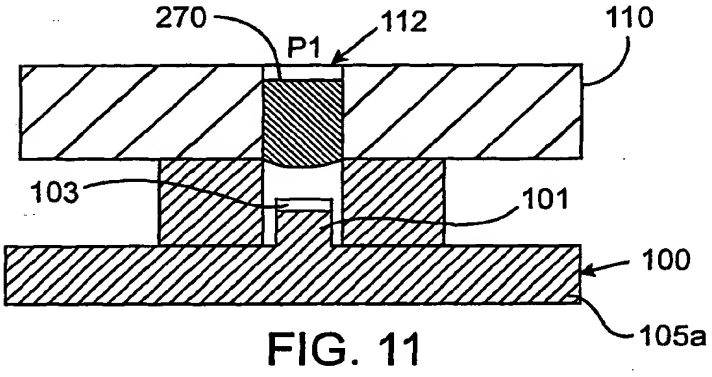


FIG. 11

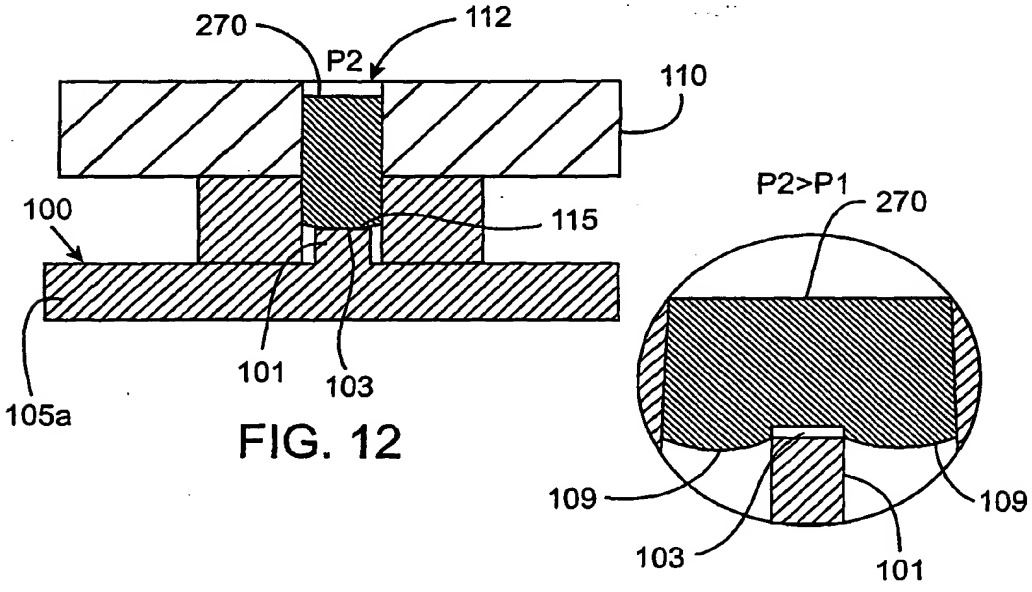


FIG. 12

FIG. 13

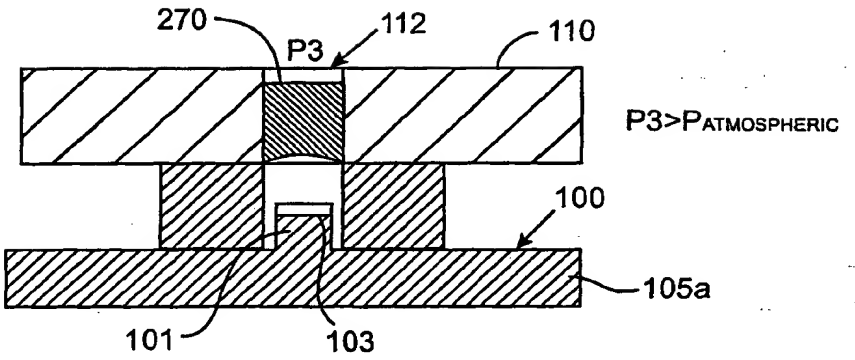
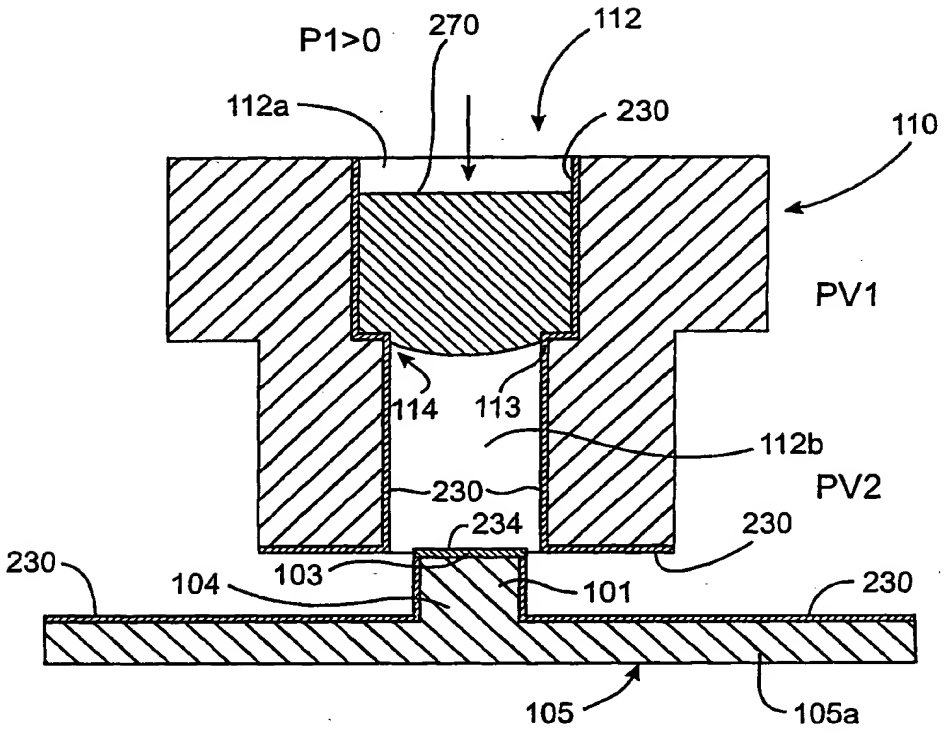


FIG. 14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112
112a
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PV1
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112b
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PV2
234
230
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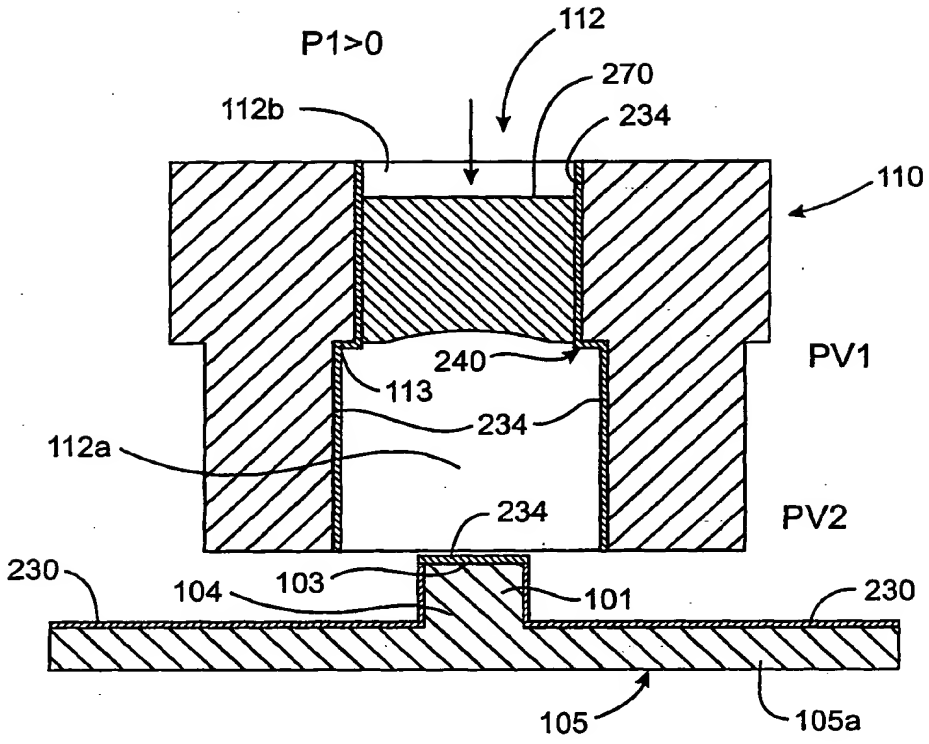
104
103
101
230
105
105a

FIG. 15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112
270
112b
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PV1
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112a
234
PV2
230
104
103
101
230
105
105a

FIG. 16

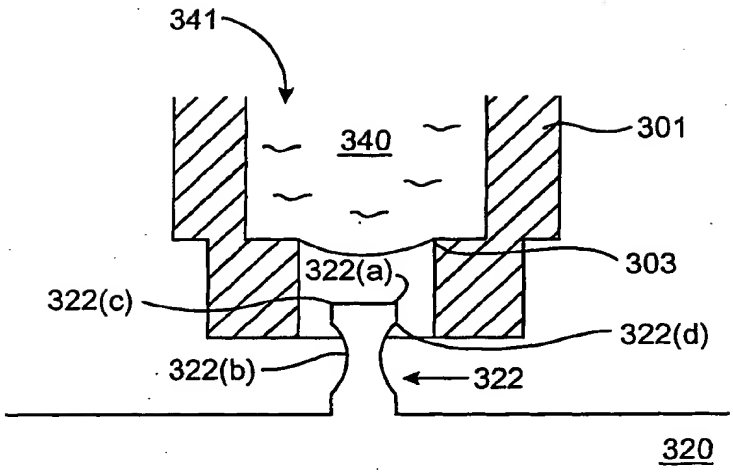

FIG. 17(a)

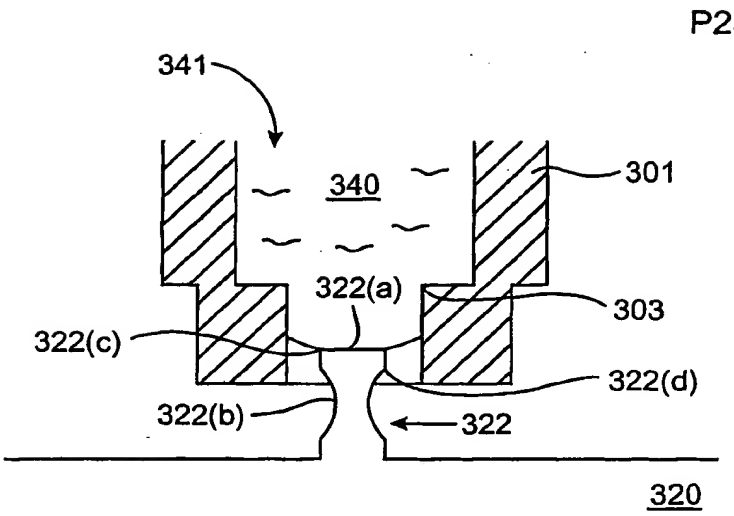

FIG. 17(b)

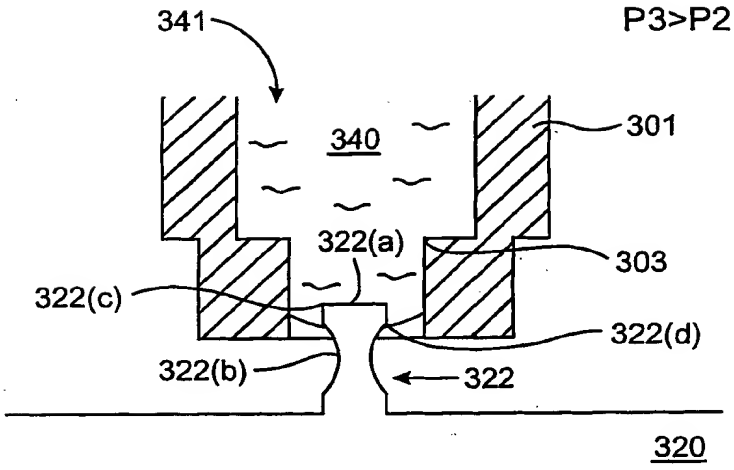

FIG. 17(c)

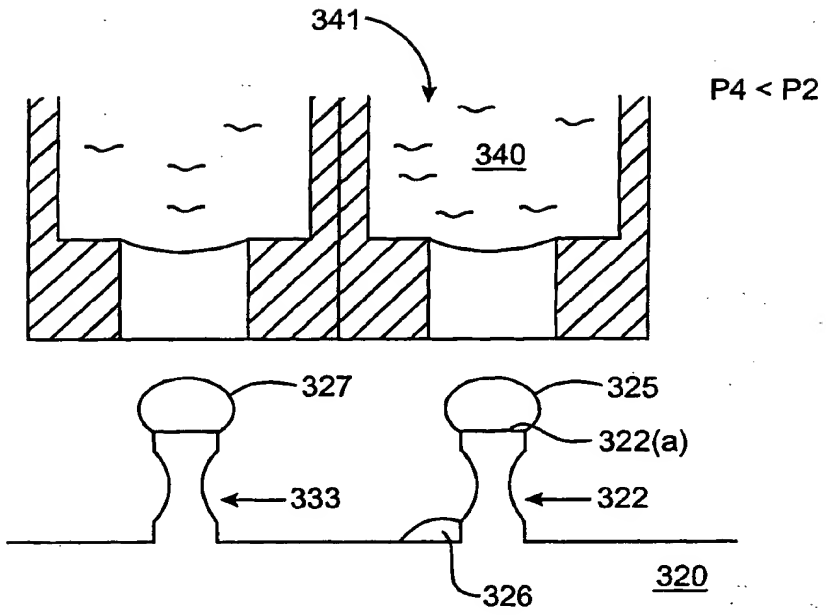

FIG. 17(d)

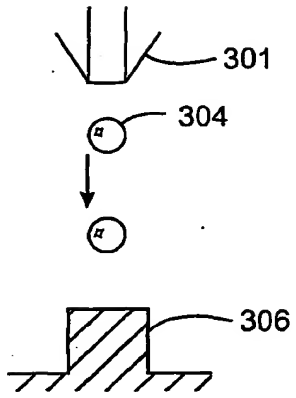

FIG. 18

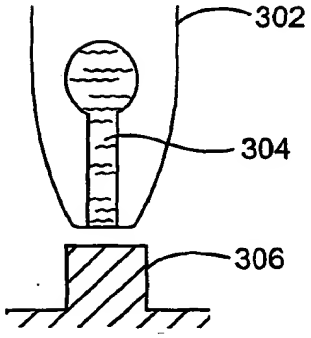

FIG. 19

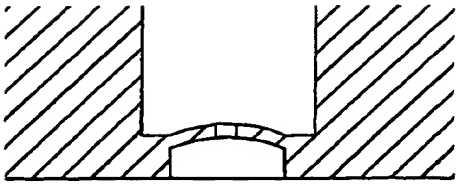

FIG. 20

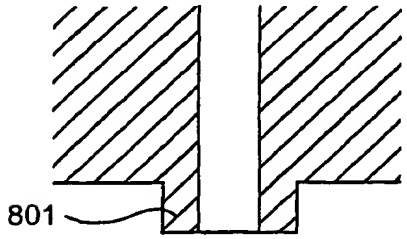

FIG. 21

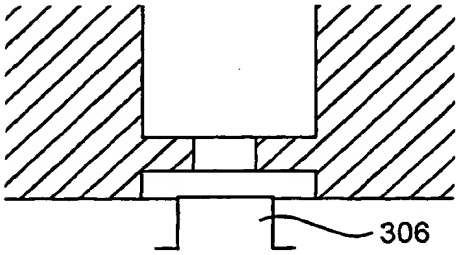

FIG. 22

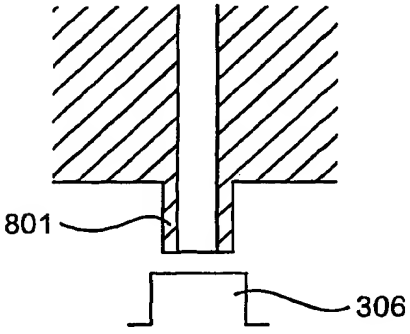

FIG. 23

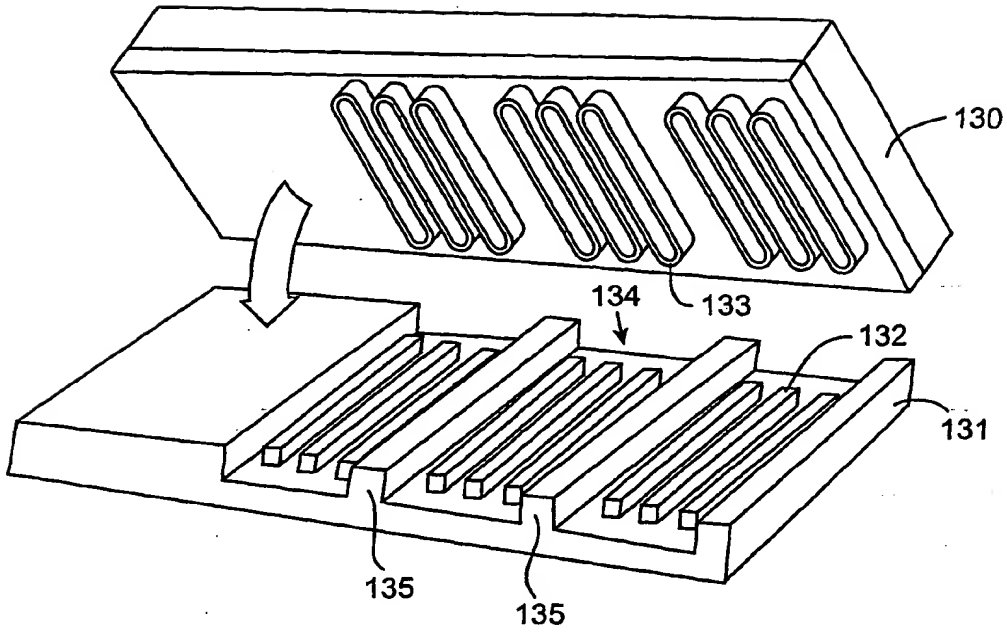

FIG. 24

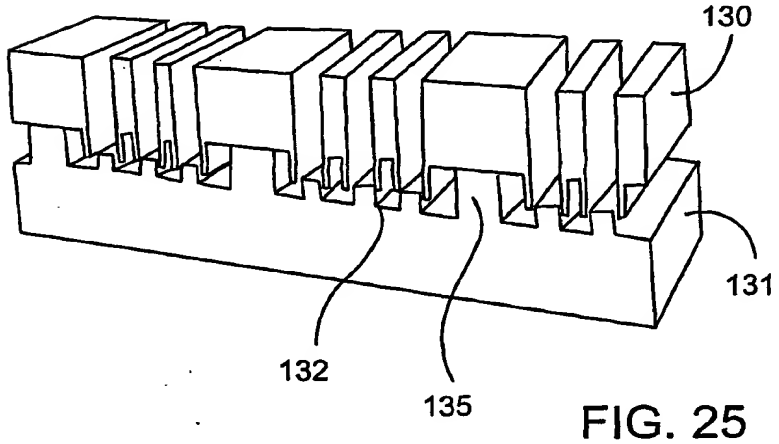

FIG. 25

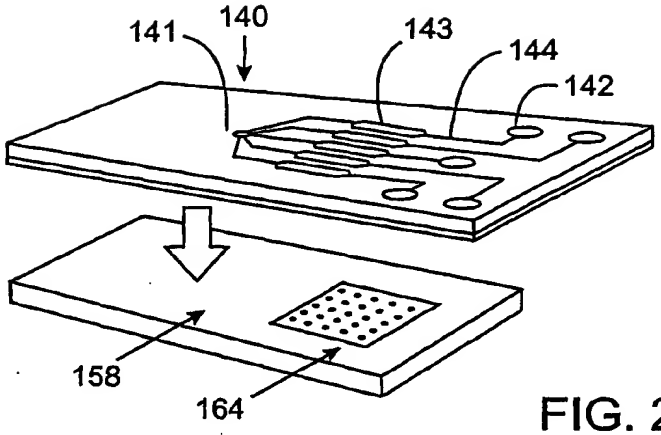

FIG. 26

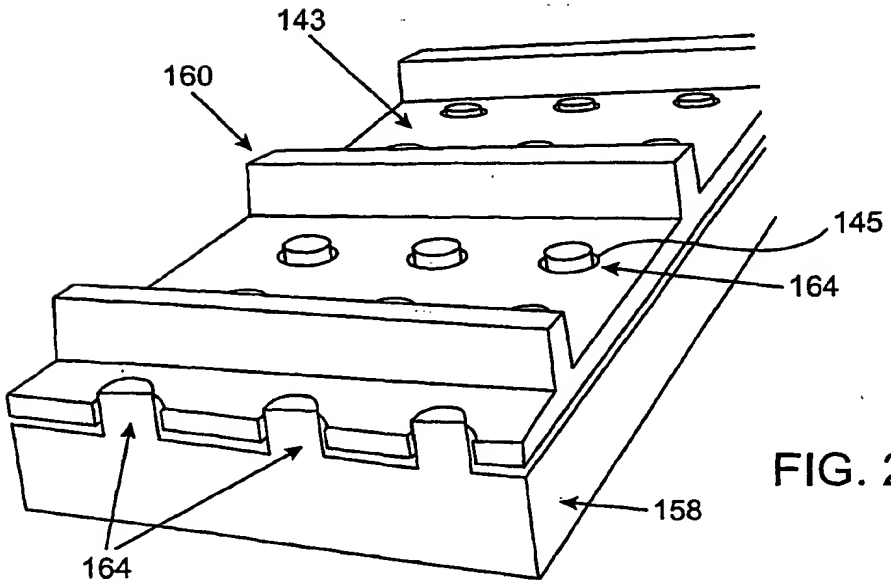

FIG. 27

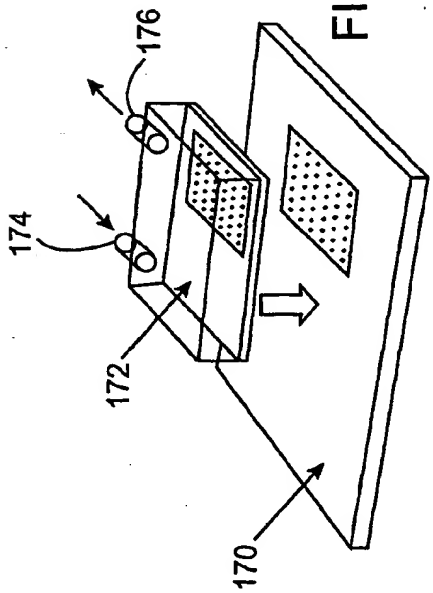

FIG. 28

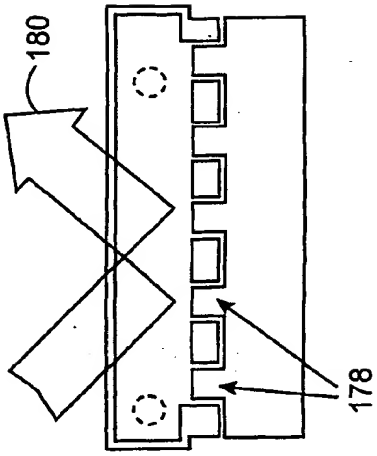

FIG. 30

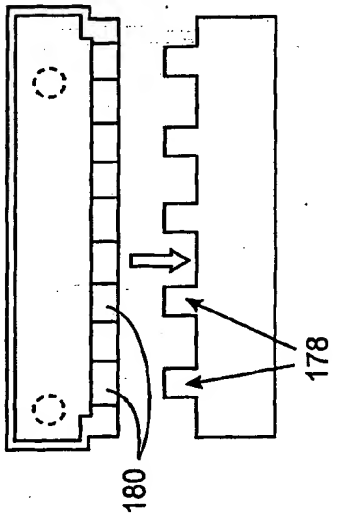

FIG. 29

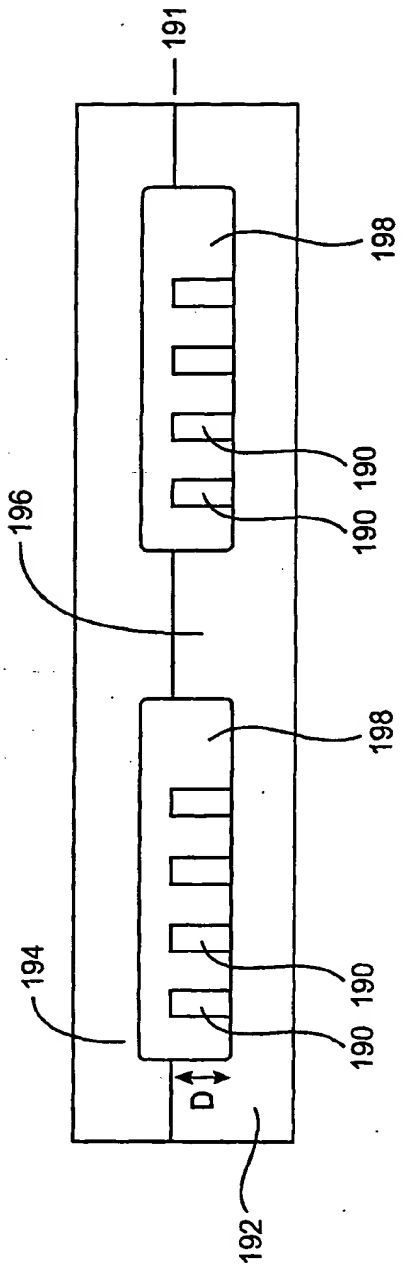

FIG. 31(a)

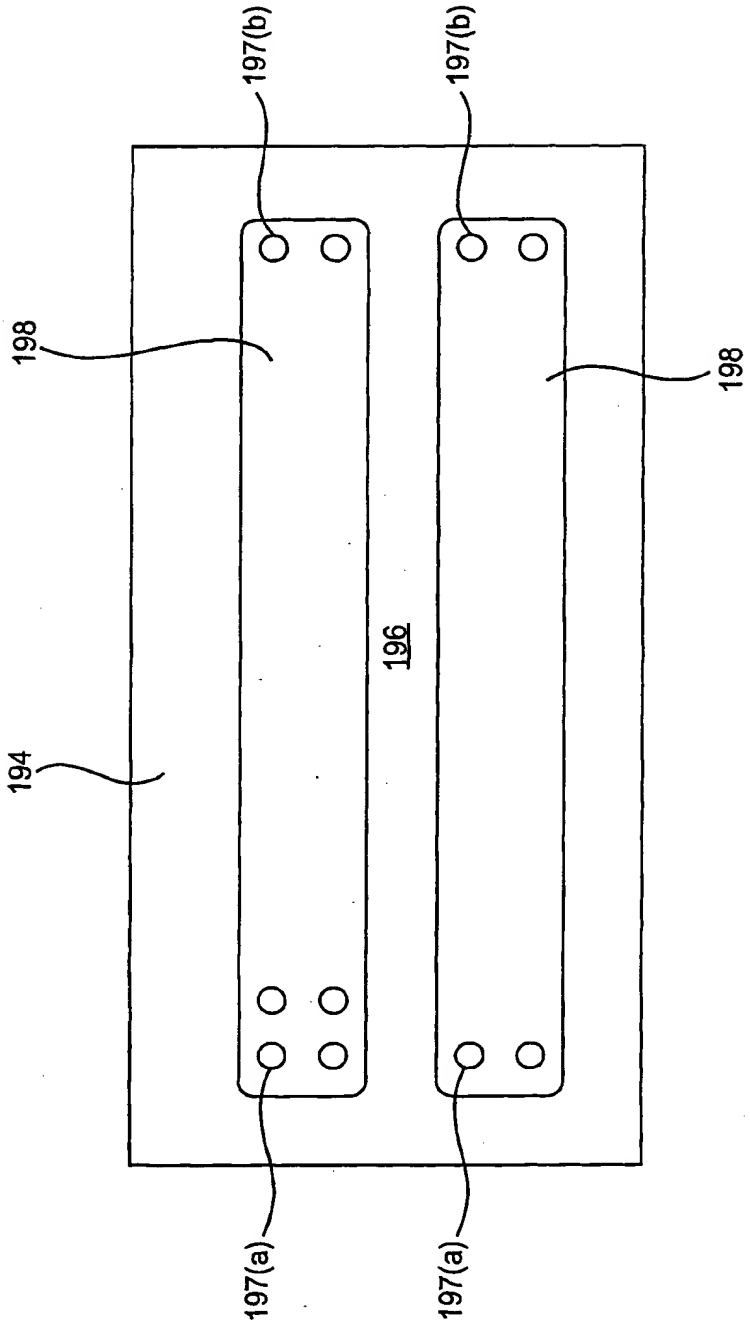

FIG. 31(b)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05966

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12M 1/18
US CL : 422/102

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/102,100,101,61:436/45-46:435/288.4,305.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 6,097,097 A (HIROSE) 01 AUGUST 2000, see figs. 4 and 5	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
28 MAY 2001	18 JUN 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LYLE A. ALEXANDER Jean Proctor Paralegal Specialist Telephone No. 703-308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05966

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

EAST
SEARCH TERMS:chip,pillar,automated analy$,(raised or projected) with surface, sample with projections,microfluidic,sample with card, nibs